Skin barrier impairment at birth predicts food allergy at two years of age

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Abstract

Background

Transcutaneous exposure to food allergens can lead to food sensitisation (FS)/food allergy (FA). We measured skin barrier function in early infancy and related it to the later development of FS/FA at 2 years.

Methods

Infants in the BASELINE birth cohort had transepidermal water loss (TEWL) measured in the early newborn period and at 2 and 6 months. At 2 years, infants had FS/FA screening using skin prick testing and oral food challenge (OFC).

Results

1903 infants were enrolled. 1355 were retained to 2 years, 1260 underwent FS screening. 6.27% had FS (79/1260, 95% CI 4.93 – 7.61%), FA prevalence was 4.45% (56/1258, 95%CI 3.38-5.74). Egg was the most prevalent allergen (2.94%), then peanut (1.75%) and cow's milk (0.74%). Day 2 upper quartile TEWL (>9$g_{water}/m^2/hr$) was a significant predictor of FA at 2 years (OR 4.1 95% CI 1.5-4.8). 75% of children with FA at 2 years had day 2 TEWL values in the upper quartile. Even in those without atopic dermatitis, infants with upper quartile day 2 TEWL were 3.5 times more likely to have FA at 2 years than infants in the lowest quartile. (CI 1.3 -11.1, p=0.04).

Conclusion

Neonatal skin barrier dysfunction predicts FA at two years, supporting the concept of transcutaneous allergen sensitisation even in infants who do not develop atopic dermatitis. TEWL could be used for stratifying infants in the first few days of life, before development of

atopic dermatitis or food allergy, for targeted intervention studies to potentially alter the atopic march.

Abbreviations

AD (Atopic Dermatitis)

BASELINE (Babies After Scope: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints)

CUMH (Cork University Maternity Hospital)

FA (Food Allergy)

FS (Food Sensitisation)

FLG (Filaggrin gene)

FLG mut (Filaggrin mutation carrier)

FLG wt (Filaggrin wild type – normal)

HPC (Household Peanut Consumption)

LTFU (Lost To Follow Up)

PA (Peanut Allergy)

SEI (Socioeconomic Index)

SpIgE (Specific Immunoglobulin E)

TEWL (Transepidermal Water Loss)

ΔTEWL (Change in TEWL measurement between one timepoint and another)

Implications

A skin barrier signal for the development of food allergy is seen in infants soon after birth, even in those infants who do not go on to develop AD.

Capsule Summary

Raised TEWL at birth can predict the development of Food Sensitisation and Food Allergy at 2 years.

Key Words

Infant,

Skin Barrier,

TEWL,

Atopic Dermatitis,

Food Sensitisation,

Food Allergy,

Prediction

Introduction

Atopic disease includes atopic dermatitis (AD), food allergy (FA), allergic rhinitis and atopic asthma.[1-4] The first manifestation of this complex disease cluster is usually AD from early infancy.[5, 6] Filaggrin (gene: *FLG*) has an important role in human skin barrier structure and function and *FLG* mutations are associated with increased risk of AD, asthma and FA, including peanut allergy, even in the absence of AD. [7-9] Immediate, IgE-mediated FA is much more prevalent in children with AD than in children without AD.[10, 11] Several lines of evidence, including murine studies support the hypothesis that percutaneous exposure to food proteins is allergenic, whereas enteral exposure is tolerogenic.[12-14] Furthermore it is suggested that peanut proteins can activate innate immune pathways in even undamaged murine skin, promoting sensitisation.[15]

The 2015 LEAP study has shown that young infants with moderate-to-severe AD are already at high risk of developing peanut allergy, which can be secondarily prevented by eating peanut regularly, compared to peanut avoidance.[16] It is possible that primary prevention could also be effective but in routine practice the pre-atopic 'at risk' population may be difficult to identify soon enough to benefit from this intervention.

Transepidermal water loss (TEWL) is a well-established measure of skin barrier disruption. TEWL is raised in those with AD at both lesional and non-lesional sites.[17, 18] It associated with *FLG* status, with increased TEWL values seen in *FLG* $_{mut}$ rather than *FLG* $_{wt}$ and with increased severity of AD.[19] We have recently shown in the BASELINE cohort, a large well phenotyped prospective birth cohort, that it is possible to use simple clinical data and TEWL to identify

newborn infants with an 80% risk of having AD at 12 months of age, compared to a background rate of 15%.[20]

Our aim in this study was to examine if early skin barrier disruption, measured by TEWL, is associated with increased rates of food sensitisation (FS - the demonstration of allergen-specific IgE in asymptomatic subjects) or clinical food allergy (FA) at two years. We hypothesised that elevated TEWL at birth would be an early risk factor for later development of FA, independent of AD.

Methods

Study subjects

This study was part of the BASELINE Birth Cohort Study (Babies After SCOPE; Evaluating Longitudinal and Nutritional Endpoints; www.baselinestudy.net), previously described in detail.[21] Infants recruited from July 2009 to October 2011 had TEWL measured at three time points in early infancy and were *FLG* genotyped. AD was screened for at 6 and 12-month appointments using the UK Working Party criteria. [22] Parental atopy was defined as a personal parental history of food allergy, AD, Rhinitis, active asthma or allergy to animals.

Skin barrier assessment

TEWL was measured using a widely validated, open-chamber system (Tewameter® TM 300; Courage+Khazaka Electronic, Cologne, Germany) [23, 24] using a standard operating procedure[25], in an environmentally controlled room, in the newborn period prior to discharge from hospital and at return visits at 2 and 6 months.

Food allergy and sensitisation

Longitudinal screening for FA was performed at each visit with screening questions about symptoms that could be attributable to a food allergen. Parents were encouraged to report any inter-appointment symptoms that they suspected to be caused by food allergy. We used modified EuroPrevall criteria to assess suspected FA. [27]

Cross-sectional screening for FA and FS was offered to all children at two years, using SPT (ALK Abello, Reading UK). The food allergen panel used for screening was cow's milk, egg, peanut, wheat, cod and soya. Other non-panel foods were tested if the child had suspected food allergic symptoms on previous ingestion of the food. Positive SPT at the two year appointment triggered a formal evaluation. If the reported food was already safely tolerated then the child was deemed *food sensitised, not allergic,* and was advised to continue to regularly eat the food. If the food had not been eaten previously, or there was a history suspicious for allergy to the food, then the child was advised to avoid the food until Oral Food Challenge (OFC) was completed (Supplemental Figure 1). Non blinded OFC were conducted, as is acceptable practice in children of this age group.[26] Criteria for stopping OFC are listed in Supplementary Table 3.

Statistical methods

Statistical analysis was carried out using SPSS (Version 21. IBM, Chicago, USA). A chi-squared test for independence was used to analyse the association between two categorical variables. The independent sample student t-test was used to compare mean score of continuous data between categorical groups such as "children with AD" versus "children without AD". Wald 95% Confidence Intervals for binomial sampling was employed. A series of univariate logistic regression (LR) models was used to estimate the factors at birth that influence the diagnosis of food allergy at 2 years. The dependent variable which measures diagnosis at 2 years is Failed Food Challenge 'Yes' =1 and 'No' = 0. All values were considered significant at $p<0.05$. Following this, a LR model was used. The dependent

variable which measures diagnosis at 2 years was again Failed Food Challenge 'Yes' =1, and 'No' = 0. All values were considered significant at $p<0.05$.

We adjusted for the following variables ($p<0.05$) in the multivariate model : 'use of emollient vs water/soap after birth'; 'type of allergen (dairy/egg vs peanut, nut, other) '; 'male vs female'; mixed feeding vs exclusive breast feeding; no visible itch vs visible itch; 2 month TEWL value. The rationale for variable selection was to include those predictors necessary for face validity but only if they were significant at a 0.05 level or they altered the coefficient of the main variable by more than 10% in cases in which the main association was significant. [28]

We used ROC (receiver operating curve) analysis to evaluate and compare the performance (sensitivity and specificity and AUC) of the LR models. Further LR was performed, selecting only for those who are NOT diagnosed with AD at 2 years, and controlling for itchy skin at 2 and 6 months, We also controlled for type of allergen (dairy/egg vs peanut, nut, other) '; 'male vs female'; mixed feeding vs exclusive breast feeding; 2 month TEWL value. The dependent variable which measures diagnosis at 2 years is Failed Food Challenge 'Yes' =1, 'No' = 0. All values were considered significant at $p<0.05$.

Ethical approval

The study was conducted according to the Helsinki guidelines. This study was approved by the Cork Teaching Hospitals Medical Ethics Committee, Cork, Ireland.

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Results

1903 infants were enrolled from July 2009 to October 2011. Demographic details are given in Supplementary Table 1. 1355 infants attended for 2 year assessment. Of these 93.9% (1260/1355) had SPT performed. Skin barrier assessment of our cohort from birth to twelve months has been previously described. [20] In brief, mean newborn TEWL was 7.32 g $_{water}/m^2/hr$ (±3.33), rising to a mean 2-month TEWL of 10.97 $g_{water}/m^2/hr$ (±7.98) where after it plateaued, with a mean 6-month TEWL of 10.71 $g_{water}/m^2/hr$ (±7.10). Filaggrin genotyping was conducted in 1300 of the cohort, with a cumulative *FLG* mutation rate (i.e. infants carrying one or more of the 4 common *FLG* mutations) of 10.46% (136/1300). At 6 months 18.7% (299/1597) of infants screened were diagnosed with AD. This decreased to 15.53% (232/1494) at 12 months and 15.9% (215/1355) at two years.

Screening for food allergy and food sensitisation at 2 years.

92.98% (1260/1355) of children who attended 2 year appointment had SPT performed. 79 of 1260 infants (6.27%; 95% CI 4.93 – 7.61%) had a positive SPT to any food; 77 infants had a positive SPT to one of the food panel and 2 positive SPT to a non-food panel food only. All positive SPTs were investigated using a structured pathway to differentiate food allergy from asymptomatic food sensitisation.

Of these 79 food sensitised infants, 22 (28%) were tolerating the food and were advised to continue its consumption. The remaining 57 infants were offered OFC. (Table 1 and Supplementary Figure 1.)

Table 1: Sensitisation and Allergy rates to Food Allergens at 2 years.

	Total Sensitised (SPT ≥ 3mm)	**Confirmed Food Allergic** (Positive food challenge or recent clinical reaction)
Total	6.27% (79/1260)	4.45% (56/1258)
Panel Foods		
Egg	3.89% (49/1260)	2.94% (37/1259)*** (95% tolerate well cooked egg)
Peanut	2.62% (33/1260)	1.75% (22/1259)***
Milk	0.95% (12/1260)	0.95% (12/1260)
Cod	0.64% (8/1257)*	0.16% (2/1260)
Wheat	0.56% (7/1259)**	0% (0/1260)
Non panel foods		
Soya	0.16% (2/1260)	0% (0/1260)
Hazelnut	0.16% (2/1260)	0.16% (2/1260)
Cashew	0.08% (1/1260)	0.08% (1/1260)
Sesame	0.08% (1/1260)	0.08% (1/1260)

**3 infants did not have SPT to Cod, ** 1 infant did not have SPT to wheat, ***1 infant sensitised to egg and 1 sensitised to peanut LTFU.*

Oral food challenge

40/41 (97%) of challenged children had positive OFC at two years. 13 children who were previously diagnosed as food allergic via OFC, did not have a repeat OFC at two years as they remained sensitised with a documented recent history of further symptomatic exposure to the food allergen. Two further infants previously diagnosed as food allergic via OFC had a negative SPT to the proven allergen, but a positive SpIgE and a documented recent history of further symptomatic exposure, so they were included in prevalence estimate for FA but not FS as demonstrated via SPT. Three infants with positive SPT but no history of safe ingestion did not complete OFC; one child had no previous reports of food allergy in earlier appointments, however experienced anaphylaxis on 1st exposure to hummus shortly before her two year appointment. Peanut screening was positive but OFC was refused by parents, the child was deemed sesame and peanut allergic (SPT Sesame 11mm, SPT peanut 7mm, SpIgE Peanut 31.2, SpIgE Ara h 2 45.5). This child is included in the prevalence estimate. Two further children with positive SPTs were lost to follow up. These children were excluded from the prevalence estimate.

The cumulative food allergy prevalence (allergic to at least one allergen) was 4.45% (56/1258) at 2 years (95% CI 3.38 – 5.74).

Non IgE mediated food allergy

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 two 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 general paediatric services for suspected Lactose Intolerance, the other two continue to have symptoms of suspected Non – IgE mediated CMPA.

Atopic Dermatitis and Food Sensitisation and Food Allergy 2 years

There was a significant association between the diagnosis of AD at any of the 3 time points and FS and FA at 2 years. For those FS at 2 years 68.4% had had a diagnosis of AD at 6 months versus those not FS at 2 years where only 16.2% had a diagnosis of AD at 6 months. This significant association between AD diagnosis and both FA and FS was seen across all 3 time points for AD diagnosis. See Supplementary Figure 2 and Supplementary Table 4.

***FLG* mutation status and Food Sensitisation and Food Allergy at 2 years**

1300 of the cohort were genotyped for the four most common loss-of-function *FLG* mutations. The cumulative *FLG* mutation rate was 10.46% (136/1300). Four infants were homozygous for *FLG* mutation, with the remainder heterozygous for a *FLG* mutation. There was a significant association between *FLG* $_{mut}$ and both FS and FA at two years. (Table 2). 981 of the 1300 infants successfully *FLG* phenotyped completed screening SPT at 2 years, with 980 successfully completing full food allergy evaluation.

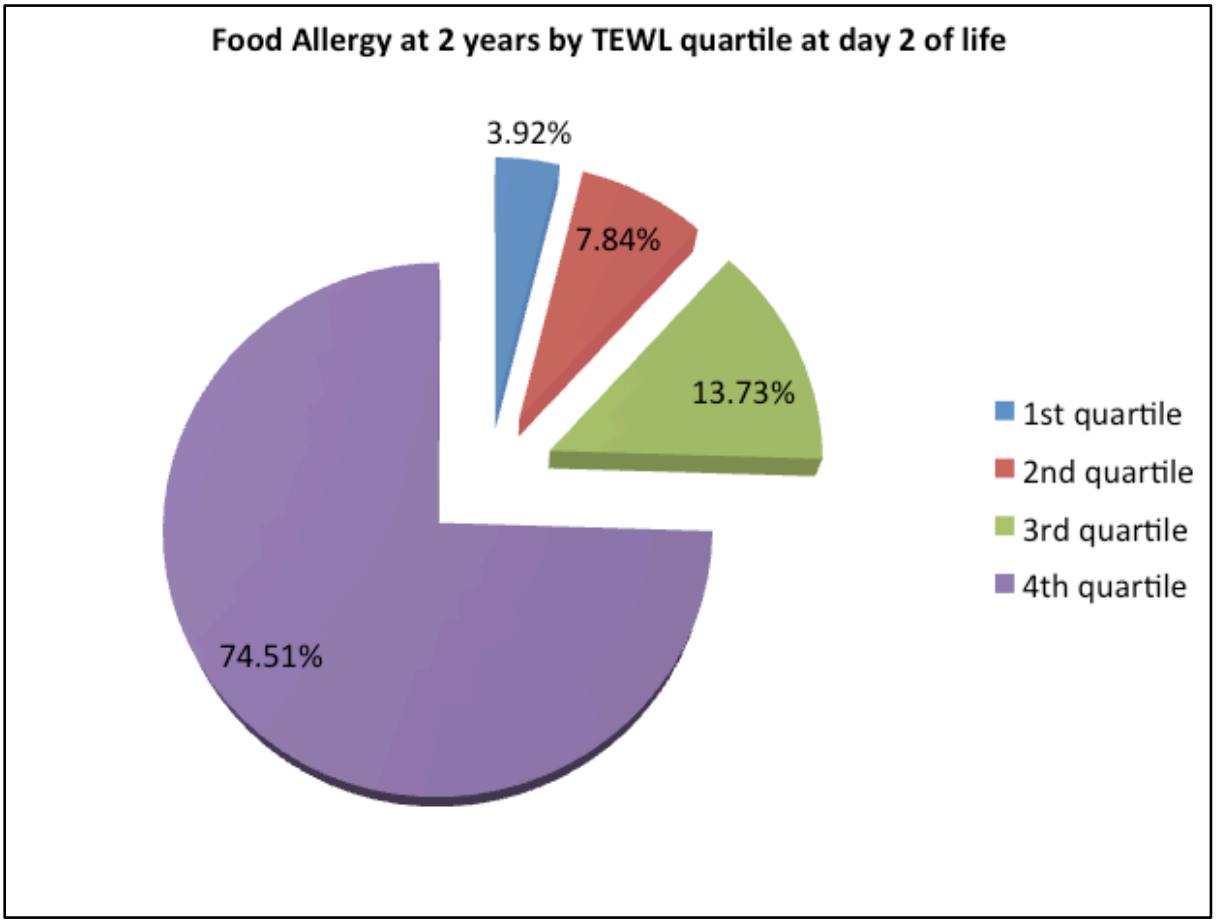
Table 2: Relationship between *FLG* and FA and FS at 2 years

	***FLG* wt**	***FLG* mut**	
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

TEWL and FS and FA at 2 years

There was no significant difference in mean TEWL values at birth between those with or without FS and FA at two years (Supplementary Table 5), however 74.5% of children with OFC proven FA at 2 years had upper quartile day 2 TEWL measurements (Figure 1). The odds ratio of having FA at 2 years, with day 2 TEWL in the top quartile vs the bottom quartile was 18.7 (CI 7.13-49.3, p <0.0001).

Figure 1. The day 2 TEWL quartile of infants with food allergy at 2 years.



Prediction of Food Allergy at Two Years

Initially, univariate logistic regression was undertaken to assess the impact of individual factors on the presence of FA at 2 year (Table 3). Infants where both parents had a history of atopy were 2.3 times more likely to have FA at 2 years than infants where neither parent had atopy (CI 1.2-4.1, p<0.01). Those with FLG_{mut} were 2.4 times more likely to have FA than FLG_{wt} (1.2-4.7, p<0.01). Mean neonatal TEWL was apportioned into quartiles; 25th, 50th, 75th at 5 $g_{water}/m^2/hr$, 7 $g_{water}/m^2/hr$ and 9 $g_{water}/m^2/hr$ respectively. Infants with neonatal TEWL in the top quartile were 3.1 times more likely to have FA at 2 years than those with lower quartile TEWL (CI 1.4-6.3, p<.004). Mean TEWL >50th centile was 2.7 times more likely to have FA at 2 years. (CI 1.3 – 5.7, p<.005)

Table 3. Univariate LR estimating factors at birth that influence FA at 2 years

Predictive factor	Odds Ratio	Confidence Intervals	P value
Birth TEWL value			
- 25 th percentile	-	-	-
	2.7	1.3-5.7	0.005*
- 50 th percentile	3.1	1.4-6.3	0.004*
- 75 th percentile			
Parental Atopy			
- No Parental Atopy	-	-	-
	1.2	0.6-2.0	0.5
- one parent	2.3	1.2-4.1	0.01*
- Both parents			
***FLG* mut (yes)**	2.4	1.2-4.7	0.01*
Male (yes)	1.2	0.8-1.8	0.2
Feeding			
- Mixed feeding	-		
	1.6	0.8-4.1	0.1
- Exclusive breast feeding	2.1	1.1-4.5	0.05*
- Exclusive formula feeding			

P = <0.05

Next, LR was conducted with any significant factors (Table 4). Infants with top quartile TEWL, *FLG* $_{mut}$ were 4.4 (CI 1.2-6.2) times more likely to have FA at 2 years than those with neither top quartile TEWL nor *FLG* $_{mut}$, with a sensitivity of 94% and specificity of 83%. When parental atopy was added to the model the sensitivity of the model remained at 93%, but specificity increased to 90%.

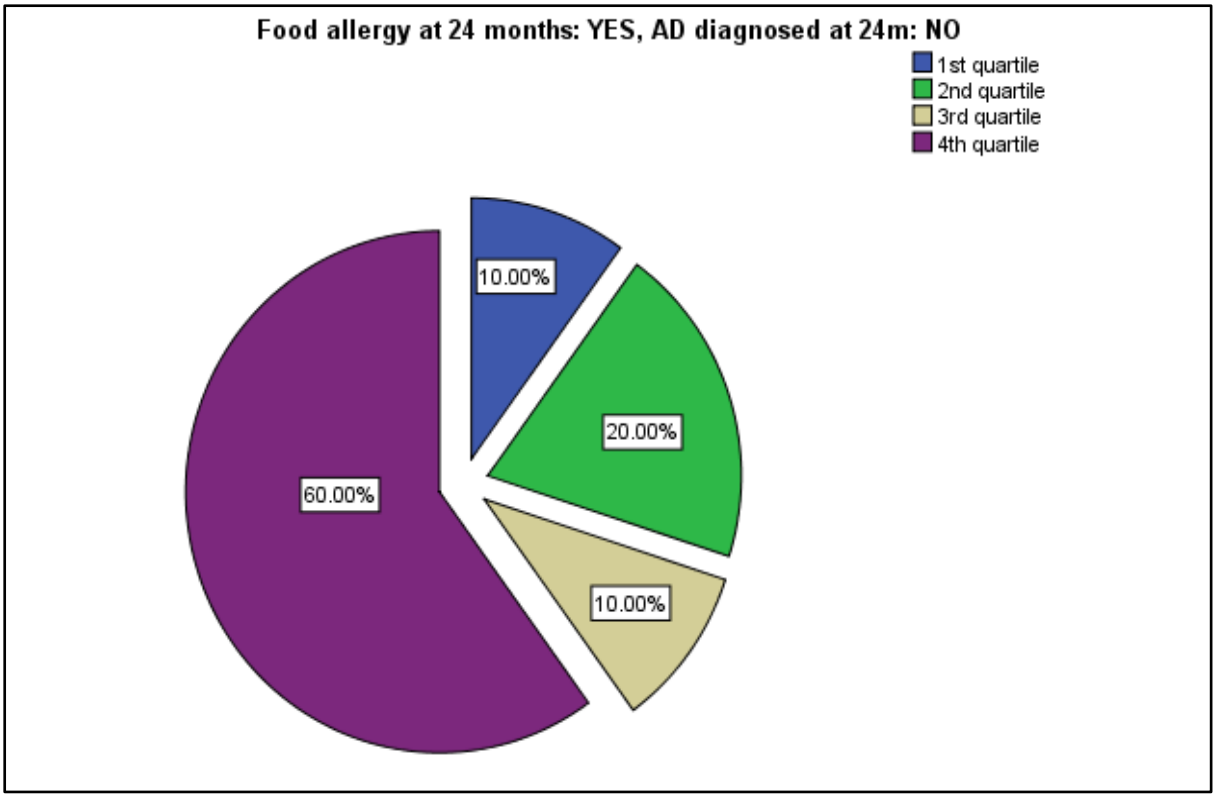
Table 4: LR model for risk factors at birth influencing diagnosis of Food Allergy at 2 years.

Predictive factor	Odds Ratio	Confidence Intervals	Sensitivity	Specificity
Top Quartile Birth TEWL	3.1	1.4-6.3	87.0	88.0
Top Quartile Birth TEWL + Parental Atopy (one parent)	4.5	1.3-6.7	90.0	89.0
Top Quartile Birth TEWL + Parental Atopy (both parents)	4.1	1.4-4.5	92.0	90.0
Top Quartile Birth TEWL + FLG	4.4	1.2-6.2	94.0	83.0
Top Quartile Birth TEWL + FLG + Parental Atopy (one parent)	5.8	3.2-9.3	93.0	90.0
Top Quartile Birth TEWL + FLG+ Parental Atopy (both parents)	4.2	2.5-7.6	93.0	92.0

Skin barrier defect without atopic dermatitis

Repeating the model selecting only for infants with FA allergy at 2 years but who did not develop AD we found that cases in the top TEWL quartile (>9 $g_{water}/m^2/hr$) who did not have AD at 2 years still remained 3.5 times more likely to be diagnosed with FA at 2 years (CI 1.3 -11.1, p=0.04) controlling for type of feeding and for parental atopy than children with TEWL in the lowest quartile neonatal TEWL.

Figure2: Food Allergy diagnosed at 2 years, with no AD by TEWL quartile.



Discussion

We have previously shown that non-invasive measures of skin barrier dysfunction in the neonatal period predate the development of atopic dermatitis at 6 and 12 months.[20] This study provides *in vivo* human data that supports evidence from prior animal studies, observational and interventional epidemiological studies that a defective skin barrier may be a route of sensitisation to food allergens. Importantly, our work shows that FS and FA occur at a higher rate in those with a neonatal barrier defect, even if the child does not develop atopic dermatitis. We have shown three quarters of children with food allergy at 2 years had day 2 TEWL values in the upper quartile and "top quartile" infants were 18 times more likely to develop food allergy than "lowest quartile" infants.

Strengths

This observational study is part of a large, prospective, antenatally and postnatally recruited and heavily phenotyped birth cohort study. It has advantages over several similar studies in that our cohort was not selected for atopy. It is the only study of this scale to assess skin barrier function in the newborn period. These infants were followed longitudinally through early infancy to two years. International criteria for diagnosis of AD and FA were used, including formal food challenge.

Limitations

Children who had dropped out of the study may have received allergy care elsewhere, leading to an underestimate of food allergy. However during the time period of this study, our centre was Ireland's only specialist paediatric allergy centre and is located in a mixed

secondary and tertiary university hospital, which was the referral centre for all food allergy cases locally, regionally and nationally. The characteristics between those still enrolled on the study at 2 years were compared to those who dropped out of the study between registration and 2 years. There was no significant difference between the mean TEWL values at any stage between those who dropped out and those still enrolled. There similarly was no significant difference in parental atopy history between both groups. (See Supplementary Table 6)

Although double-blind, placebo-controlled OFC is the gold standard for food allergy diagnosis, we performed only open OFCs. In recent years this has become an acceptable standard practice in the age group we studied.[26] Infants with non-IgE mediated allergy to foods and food intolerances may have been omitted or overlooked. However our frequent follow up and study appointments revealed consistent results with other studies whereby, most infants who had been avoiding foods were able to reintroduce them before 2 years of age. [29]

We have demonstrated for the first time an association between early life TEWL and FA and FS at 2 years. At birth, a higher quartile TEWL was significantly predictive of FA at two years, with three quarters of FA children having had top quartile day 2 TEWL. Though the relationship between AD and FA has been previously shown, this signal at birth obviously and importantly precedes the development of atopic dermatitis, giving indirect evidence for the initiation of food allergen sensitisation across a skin barrier defect in humans. Critically, this effect is present even in the absence of AD, which shows that, although the skin barrier may not be symptomatic or obviously deficient or abnormal on clinical examination,

functional deficiency of the skin barrier can be present, leading to sensitisation and possibly clinical food allergy even in those without active clinical atopic dermatitis.

Some children with a defective skin barrier develop sensitisation to food allergens but are clinically tolerant, while others develop sensitisation and become food allergic. This phenomenon, while well recognised, is currently poorly understood but may be due to environmental factors, such as food antigen levels in their home environment [30], timing of ingestion of food [16] or other environmental factors, including barrier stressors such as soaps, detergents and environmental exposures.

Detection of neonates who are already, in the first few days of life, at higher risk of development of AD and FA, prior to clinical expression of symptoms of either AD or FA, is a novel and unique finding in the general paediatric population. Recent progress has been made in the field of allergy prevention with strategies emerging regarding optimum timing of introduction of food allergens that may represent secondary prevention of FA in children who have already developed AD. Development of oral tolerance may be facilitated by early introduction and ingestion of the food antigen.[16]

Importantly until there are randomised control trials demonstrating that the detection of impaired skin barrier at birth, and the subsequent protection of this barrier can prevent allergy TEWL measurement at birth should be confined to the research setting. The implantation of screening prior to this would inevitably lead to parental anxiety without offering parents an effective solution or disease modifying agent to halt the potential disease process.

Along with an increased understanding of the early mechanisms in AD arising from genetic advances in AD, recently published pilot intervention studies show primary prevention of AD itself may be possible by use of liberal emollients from the first 3 weeks of life to 6 months of age.[31, 32] Our findings relating to neonatal TEWL and atopic dermatitis suggest that this intervention might have to start even earlier - in the first few days of life - as we have shown an abnormal TEWL signal is already present at birth. Such an intervention may also attenuate transepidermal sensitisation to foods. A combination of neonatal TEWL screening, skin barrier protection in highest risk infants and early introduction of dietary allergens may change the profile of the allergic diseases that represent such medical, financial and social burdens for affected families around the world.

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References

1. Johansson SGO, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004; **113**(5): 832-6.
2. Venter C, Hasan Arshad S, Grundy J, et al. Time trends in the prevalence of peanut allergy: three cohorts of children from the same geographical location in the UK. *Allergy* 2010; **65**(1): 103-8.
3. Duggan EM, Sturley J, Fitzgerald AP, Perry IJ, Hourihane JOB. The 2002–2007 trends of prevalence of asthma, allergic rhinitis and eczema in Irish schoolchildren. *Pediatr Allergy Immunol* 2012; **23**(5): 464-71.
4. Prescott SL, Pawankar R, Allen KJ, et al. A global survey of changing patterns of food allergy burden in children. *World Allergy Organisation Journal* 2013; **6**(21).
5. Illi S, von Mutius E, Lau S, et al. The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. *J Allergy Clin Immunol* 2004; **113**(5): 925-31.
6. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. *J Allergy Clin Immunol* 2003; **112**(6): S118-S27.
7. Irvine AD, McLean WHI, Leung DYM. Filaggrin Mutations Associated with Skin and Allergic Diseases. *New England Journal of Medicine* 2011; **365**(14): 1315-27.
8. McAleer MA, Irvine AD. The multifunctional role of filaggrin in allergic skin disease. *J Allergy Clin Immunol* 2013; **131**(2): 280-91.
9. Brown SJ, Asai Y, Cordell HJ, et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. *J Allergy Clin Immunol* 2011; **127**(3): 661-7.
10. Sampson HA, McCaskill CC. Food hypersensitivity and atopic dermatitis: Evaluation of 113 patients. *The Journal of Pediatrics* 1985; **107**(5): 669-75.
11. Eigenmann PA, Sicherer SH, Borkowski TA, Cohen BA, Sampson HA. Prevalence of IgE-Mediated Food Allergy Among Children With Atopic Dermatitis. *Pediatrics* 1998; **101**(3): e8.
12. Strid J, Hourihane J, Kimber I, Callard R, Strobel S. Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. *Clin Exp Allergy* 2005; **35**(6): 757-66.
13. Strid J, Thomson M, Hourihane J, Kimber I, Strobel S. A novel model of sensitization and oral tolerance to peanut protein. *Immunology* 2004; **113**(3): 293-303.
14. Strid J, Hourihane J, Kimber I, Callard R, Strobel S. Disruption of the stratum corneum allows potent epicutaneous immunization with protein antigens resulting in a dominant systemic Th2 response. *European journal of immunology* 2004; **34**(8): 2100-9.
15. Tordesillas L, Goswami R, Bened, et al. Skin exposure promotes a Th2-dependent sensitization to peanut allergens. *The Journal of Clinical Investigation* 2014; **124**(11): 4965-75.
16. Du Toit G, Roberts G, Sayre PH, et al. Randomized Trial of Peanut Consumption in Infants at Risk for Peanut Allergy. *New England Journal of Medicine* 2015; **372**(9): 803-13.
17. Cork MJ, Danby SG, Vasilopoulos Y, et al. Epidermal Barrier Dysfunction in Atopic Dermatitis. *The Journal of investigative dermatology* 2009; **129**: 1892-908.
18. Seidenari S GG. Objective assessment of the skin of children affected by atopic dermatitis: a study of pH, capacitance and TEWL in eczematous and clinically uninvolved skin. *Acta Derm Venereol* 1995; **75**(6): 429-33.
19. Flohr C, England K, Radulovic S, et al. Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age. *British Journal of Dermatology* 2010; **163**(6): 1333-6.
20. Kelleher M, Dunn-Galvin A, Hourihane JOB, et al. Skin barrier dysfunction measured by transepidermal water loss at 2 days and 2 months predates and predicts atopic dermatitis at 1 year. *J Allergy Clin Immunol*.
21. O'Donovan SM, Murray DM, Hourihane JOB, Kenny LC, Irvine AD, Kiely M. Cohort profile: The Cork BASELINE Birth Cohort Study: Babies after SCOPE: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints. *International Journal of Epidemiology* 2014.

22. Williams HC BP, Hay RJ, Archer CB, Shipley MJ, Hunter JJ, Bingham EA, Finlay AY, Pembroke AC, Graham-Brown RA, et al. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *The British journal of dermatology* 1994; **131**(3): 383-96.
23. Fluhr JW, Akengin A, Bornkessel A, et al. Additive impairment of the barrier function by mechanical irritation, occlusion and sodium lauryl sulphate in vivo. *British Journal of Dermatology* 2005; **153**(1): 125-31.
24. Barel AO CP. Study of the stratum corneum barrier function by transepidermal water loss measurements: comparison between two commercial instruments: Evaporimeter and Tewameter. . *Skin pharmacology and physiology* 1995; **8**(4): 186-95.
25. Kelleher MM, O'Carroll M, Gallagher A, et al. Newborn Transepidermal Water Loss Values: A Reference Dataset. *Ped Derm* 2013
26. Boyce J AaA, Burks AW, Jones SM, Sampson HA, Wood RD et al. Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel. J Allergy Clin Immunol 2010; 126(6): S1-S58.
27. Keil T, McBride D, Grimshaw K, et al. The multinational birth cohort of EuroPrevall: background, aims and methods. *Allergy* 2010; **65**(4): 482-90.
28. Budtz–Jørgensen E, Keiding N, Grandjean P, Weihe P. Confounder Selection in Environmental Epidemiology: Assessment of Health Effects of Prenatal Mercury Exposure. *Annals of Epidemiology* 2007; **17**(1): 27-35.
29. Schoemaker AA, Sprikkelman AB, Grimshaw KE, et al. Incidence and natural history of challenge-proven cow's milk allergy in European children –EuroPrevall birth cohort. *Allergy* 2015
30. Brough HA, Liu AH, Sicherer S, et al. Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. *J Allergy Clin Immunol* 2015; **135**(1): 164-70.e4.
31. Simpson EL, Chalmers JR, Hanifin JM, et al. Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *J Allergy Clin Immunol* 2014; **134**(4): 818-23.
32. Horimukai K, Morita K, Narita M, et al. Application of moisturizer to neonates prevents development of atopic dermatitis. *J Allergy Clin Immunol*; **134**(4): 824-30.e6.

Supplementary Tables:

Supplementary Table 1: Baseline Demographics

	N= 1903
Total enrolled	1903
Male/female %	50.4% /49.6%
Birthweight mean (+/- SD)	3489 gms (±512gms)
Gestation(mean (+/- SD)	279.33 days (±10.77)

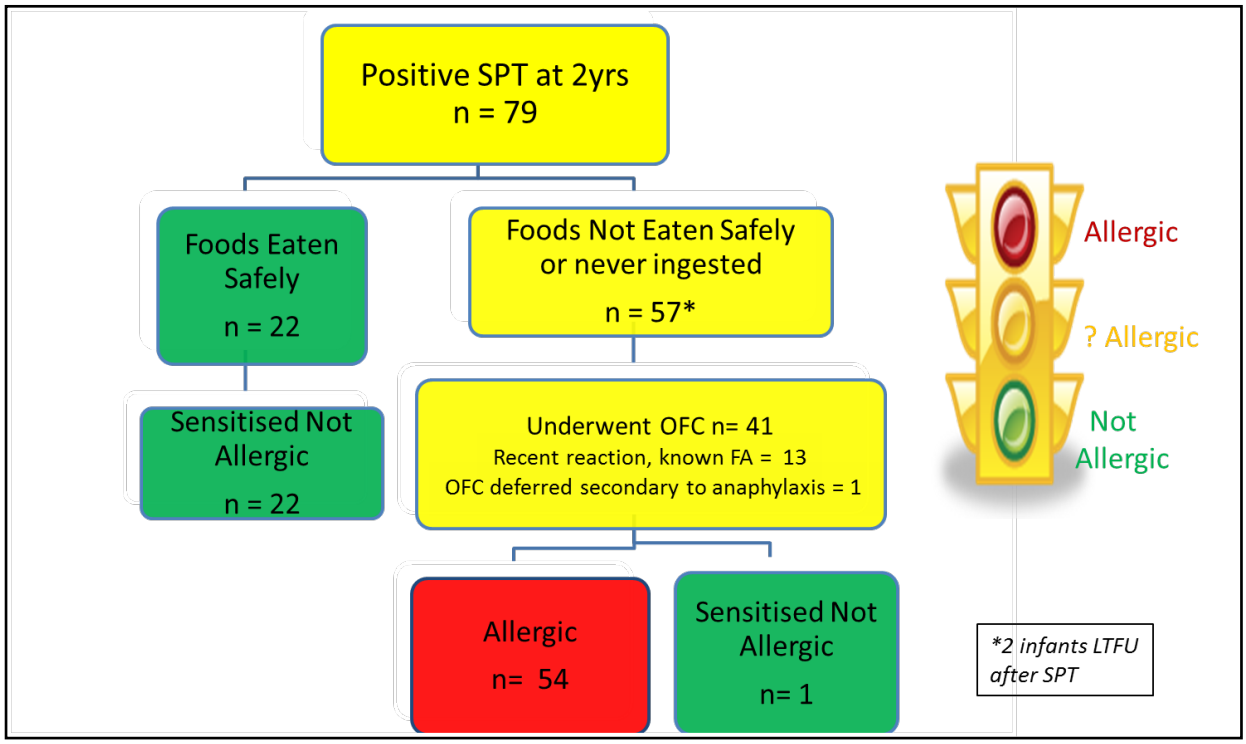
Supplementary Table 2: Number of infants at 2 years with TEWL measurements

	Total enrolled	**Attended at 2 years**	**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, 2m, 6m)	1292	1110	1030

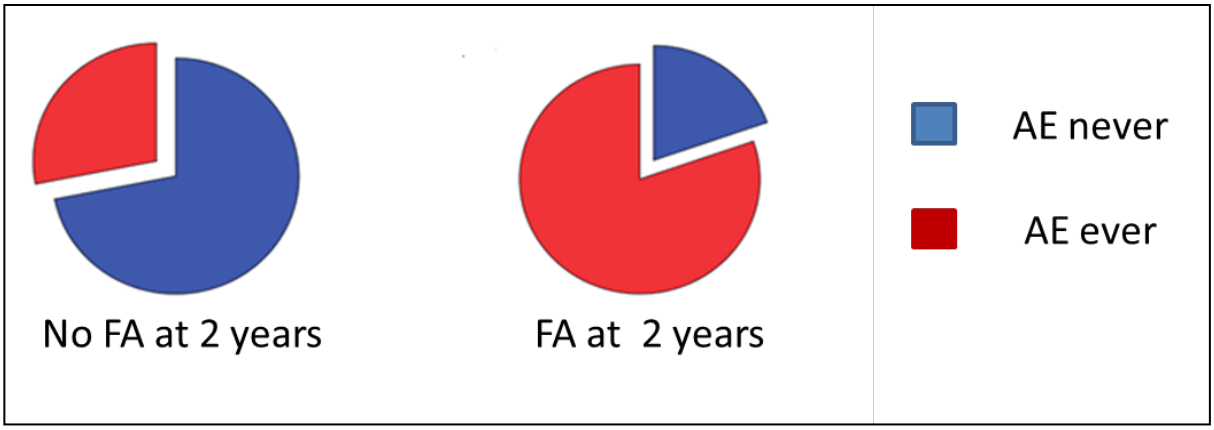
Supplementary Table 3. Criteria for positive OFC

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

Supplementary Figure 1: Investigation of positive SPT at 2 years.



Supplementary Figure 2: Relationship between AE at any time point and FA at 2 years



Supplementary Table 4: Relationship between AD at any time point and FA and FS at 2 years

	FS	**Not FS**	**p value**	**FA**	**Not FA**	
N	*79*	*1181*		*56*	*1202*	*(2 LTFU)*
AE 6 mth	54/79 (68.4%)	184/1138 (16.2%)	p = .000	40/56 (71.4%)	199/1190 (17.2%)	p = .000
AD 12 mth	48/79 (60.8%)	146/1161 (12.6%)	p = .000	38/55 (69.1%)	156/1183 (13.2%)	p = .000
AD 2 years	44/79 (56.4%)	166/1181 (14.1%)	p = .000	34/56 (60.7%)	176/1202(14.6%)	p = .000

**some infants did not attend all clinical appointments*

Supplementary Table 5: TEWL values ($g/_{water}/m^2$) vs FS and FA at 2 years

	FS	Not FS	p value	FA	Not FA	p value
N	*79*	*1181*		*56*	*1202*	*(2LTFU)*
Birth	6.70 (±2.87)	7.38 (±3.3)	0.088	6.49 (±2.69)	7.38 (±3.3)	0.06
2 mths	12.88 (±8.78)	10.93 (±8.12)	0.04*	12.83 (±9.64)	10.98 (±8.1)	0.1
6 mths	15.37 (±10.84)	10.32 (±6.62)	0.00**	16.29 (±11.83)	10.39 (±6.65)	0.00**
Δ Birth – 2 mths	6.36 (±9.48)	3.52 (±8.44)	0.007**	6.43 (±10.47)	3.59 (±8.45)	0.02*
Δ Birth – 6 mths	8.13 (±10.88)	2.88 (±7.22)	0.00**	8.98 (±11.67)	2.96 (±7.25)	0.00**
Δ 2 – 6 mths	1.81 (±11.59)	-.61 (±9.92)	0.044*	2.65 (±12.48)	-.6 (±9.9)	0.02*

**p< 0.05 **p< 0.05*

Supplementary Table 6: Comparison of Characteristics between enrolled infants and drop outs

	Enrolled at 2 years	Drop outs	
Total (n)	1355	548	
Mean TEWL ($g/_{water}/m^2/min$)			
- Birth	7.35 (±3.31)	7.25 (±3.42)	0.58
- 2 month	11.07 (±8.22)	10.62 (±6.98)	0.32
- 6 month	10.66 (±7.04)	11.01 (±7.47)	0.51
Birth Weight (gms)	3511.2 (±512)	3435.8 (±509)	0.004*
Parental history of atopy	65.4% (854/1305)	60.6% (214/353)	0.09
Socioeconomic Index	43.1 (±15.68)	40.2 (±16.56)	0.002*