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Atopic dermatitis and disease severity are the main risk factors for food sensitization in exclusively breastfed infants

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Abstract

Filaggrin loss-of-function (*FLG*) skin barrier gene mutations are associated with atopic dermatitis (AD) and transepidermal water loss (TEWL). We investigated whether *FLG* mutation inheritance, skin barrier impairment and AD also predispose to allergic sensitization to foods. 619 exclusively breastfed infants were recruited at 3 months of age and examined for AD and disease severity (SCORAD) and screened for the common *FLG* mutations. TEWL was measured on unaffected forearm skin. In addition, skin prick testing to six foods (cow's milk, egg, cod, wheat, sesame, and peanut) was performed. Children with AD were significantly more likely to be sensitized (adjusted OR=6.18 (95% CI 2.94-12.98, p<0.001), but this effect was independent of *FLG* mutation carriage, TEWL and AD phenotype (flexural vs non-flexural). There was also a strong association between food sensitization and AD severity (adjusted $OR_{SCORAD<20}$=3.91 (1.70-9.00, p=0.001) vs adjusted $OR_{SCORAD\geq20}$=25.60 (9.03-72.57), p<0.001). Equally, there was a positive association between AD and sensitization to individual foods (adjusted OR_{egg}=9.48 (3.77-23.83), p<0.001; adjusted $OR_{cow's\ milk}$=9.11 (2.27-36.59), p=0.002; adjusted OR_{peanut}=4.09 (1.00-16.76), p=0.05). AD is the main skin-related risk factor for food sensitization in young infants. In exclusively breastfed children this suggests that allergic sensitization to foods can be mediated by cutaneous antigen-presenting cells.

INTRODUCTION

The recent discovery of the common loss-of-function variants in the *FLG* gene, encoding the epidermal barrier protein filaggrin, and the strong association with atopic disease have led to a heightened interest in the role of skin barrier dysfunction in the development of AD, allergic sensitization and also respiratory allergies (Smith et al., 2006; Palmer et al., 2006; Sandilands et al., 2007; Sandilands et al., 2009; Irvine et al., 2012; Boralevi et al., 2008).

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The current hypothesis is that in individuals without a skin barrier defect, there is full integrity of the epidermis marked by minimal transepidermal water loss and adequate protection against environmental insults, such as microbes and environmental allergens. However, where people carry a skin barrier gene mutation, such as a loss-of-function mutation in the *FLG* gene, transepidermal water loss is increased. Probably in interaction with environmental factors, such as frequent use of protease-containing detergents and soaps, the integrity of the skin barrier is gradually broken down, and this may lead to the typical immunological changes seen in eczematous skin (Cork et al., 2006). Animal work suggests that environmental allergens, such as house dusts mites but also food protein, can make contact with the immune system via antigen-presenting cells in the superficial epidermis, leading to sensitization, which could potentially make existing AD worse and may also be an important precursor of food and respiratory allergies (Fallon et al., 2009; Lack et al., 2003). This would explain why *FLG* mutations are only associated with asthma in the presence of AD and/or allergic sensitization (van den Oord and Sheikh, 2009). However, none of the above has been demonstrated in a prospective study setting, and therefore the exact sequence of events remains uncertain.

At present, we only have evidence from studies among older children and adults, which suggests that those who carry a *FLG* skin barrier gene mutation have a higher risk of developing AD, early disease onset and more severe disease compared to wild type children (Nemoto-Hasebe et al., 2009; Barker et al., 2007; Brown et al., 2008; Ezzedine et al., 2012).

We have recently shown that *FLG* mutation carriage is associated with early onset AD. Carrying at least one *FLG* mutation was also significantly associated with raised transepidermal water loss (TEWL), even in the absence of AD, suggesting that skin barrier impairment may be an important precursor of eczematous skin inflammation (Flohr et al., 2010). Furthermore, we have demonstrated a positive association between *FLG* mutation carriage, parental report of AD symptoms and challenge-proven peanut allergy in school children (Brown et al., 2011). The association between AD and peanut allergy remained significant even after adjustment for *FLG* status, which suggests that eczematous skin inflammation can act as a mediator of food sensitization. However, early food sensitization, AD severity and TEWL were not assessed in this cohort.

We therefore tested the hypothesis that inheritance of *FLG* mutations, skin barrier impairment (raised TEWL) and AD increase the risk of food sensitization in a cohort of 3 month old infants who were all exclusively breastfed.

RESULTS

The association between AD, *FLG*, and TEWL

24.9% (154/619) of children had clinical AD, with a median SCORAD of 7.6 (range 3.5-71.5). 132/154 children (85.7%) had mild AD (SCORAD <20) and 22/154 (14.3%) had moderate to severe disease (SCORAD ≥20). Median TEWL [gram/(m^2×h)] in infants without AD was 12.18 (IQR 10.24-14.96) and significantly lower compared to affected children, 15.41, IQR 12.30-21.83, p<0.001. Higher TEWL was associated with more severe disease, r=0.55 (p<0.001), median TEWL 'SCORAD <20' 14.7, IQR 12.2-18.8 vs 'SCORAD ≥20' 26.4, IQR 15.4-39.7, p<0.001. In our children, 12.1% (75/619) carried at least one *FLG* mutation, of which 70 (93.3%) were heterozygotes and 5 (6.7%) either homozygous or compound heterozygotes. The prevalence of *FLG* mutations increased to 24.0% (37/154) among children with AD. *FLG* mutation carriers were more likely to have AD by three months of age (OR=3.55, 2.16-5.84, p<0.001). In addition, *FLG* mutations were significantly associated with higher median TEWL: all children *FLG* 'yes' 15.77, IQR 12.31-25.52 vs *FLG* 'no' 12.43, IQR 10.46-15.38, p<0.001. This effect was true even in the

absence of clinical AD: *FLG* 'yes' 14.15, IQR 12.16-16.81 vs *FLG* 'no' 11.94, IQR 10.19-14.77, p<0.001.

The association between AD, *FLG*, TEWL, and food sensitization

With regard to food sensitization, 5.5% of children (34/619) were sensitized to one of the six study foods (SPT ≥1mm), or 4.0% (25/619) using 3mm as the skin prick test diameter cut off. Egg white sensitization was commonest (n=24), followed by cow's milk (n=10), peanut (n=8), and wheat (n=2). None of the children were sensitized to cod fish or sesame. *FLG* mutations were not significantly associated with food sensitization (adjusted OR all children=1.21, 0.45-3.24, p=0.70 (Table 1); adjusted OR children with AD=1.48, 0.46-4.75 vs adjusted OR children without AD=1.18, 0.15-9.40). There was a significant association between TEWL and food sensitization (TEWL < vs >15 gram/(m^2×h); adjusted OR=3.26, 1.61-6.62, p=0.001; Table 1). This remained significant when adjusted for both *FLG* mutation status and AD presence, but became non-significant following adjustment for AD severity (Table 3). AD presence and severity were themselves strongly associated with food sensitization (Table 1, SPT ≥1mm). The results became even more significant when a skin prick test diameter cut off of 3mm was used (adjusted OR=8.53, 3.51-20.65, p<0.001; adjusted $OR_{SCORAD<20}$=4.44, 1.60-12.34, p=0.004; and adjusted $OR_{SCORAD\geq20}$=45.77, 14.47-144.81, p<0.001). Similar associations were found for individual sensitization to egg, cow's milk, and peanut (Table 2; SPT ≥1mm), and the 3 mm cut off risk estimates were as follows: adjusted OR_{egg}=9.29 (3.43-25.18), p<0.001; adjusted $OR_{cow's\ milk}$=11.14 (2.17-57.18), p=0.004; adjusted OR_{peanut}=7.87 (0.70-88.20), p=0.09. Although AD phenotypye (no AD vs flexural vs non-flexural vs *both* flexural and non-flexural) showed a highly significant stepwise increase in food sensitization risk, this was mediated by disease severity (SCORAD) rather than being specific to the disease site (Table 1).

DISCUSSION

We have shown that early onset AD and disease severity as well as skin barrier impairment (TEWL) are associated with the risk of food sensitization at 3 months of age, while *FLG* mutation carriage was not an independent risk factor. AD phenotype was acting as a proxy for AD severity.

To the best of our knowledge, the association between *FLG* mutations, AD, disease severity, disease phenotype and food sensitization during the first three months of life has not been previously reported. A strength of our study is that we physically examined all children for AD, rather than relying on parental report of a doctor diagnosis. In addition, we measured AD severity and TEWL as a measure of phenotypic skin barrier impairment. Our data suggests that this was a sensitive outcome, given that we detected significant TEWL differences in association with *FLG* mutations even in the absence of clinical AD. We were also able to examine the effect of AD and disease severity on individual food allergens.

However, it is important to keep in mind that food sensitization does not always equate to clinical allergy and longitudinal follow up of our cohort is underway, which will include double-blind placebo-controlled food challenges. In addition, we only performed skin prick testing, rather than specific IgE measurements, but the former is considered to be a more specific predictor of clinical food allergy in later life (Osborne et al., 2011).

As all our study children were exclusively breastfed and food protein levels are relatively low in breastmilk (COT Statement UK Department of Health, 2008), it is possible that food sensitization was mediated via antigen-presenting cells in the skin rather than the gut. This concept is supported by our observation that the topical use of peanut-oil increases the risk of peanut allergy, in particular in children with a history of AD (Lack et al., 2003). In

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addition, our case-control study on the association between AD and peanut allergy in older children suggested a seven times increased risk of challenge-proven peanut allergy between 1 and 18 years of age in association with a history of AD between 6 and 42 months (adjusted OR=7.4, 4.1-13.7), even when *FLG* mutation carriage status was taken into account (Brown et al., 2011). Interestingly, in the same study we also found a significant independent risk increase in peanut allergy in association with carriage of a *FLG* loss-of-function mutation (OR=3.8, 1.7-8.3) but no measure of skin barrier impairment was included and adjusted for in the analysis.

Although there is mounting evidence for percutaneous sensitization in individuals with inflamed skin, and experimental evidence for increased percutaneous sensitization in filaggrin-deficient mice, the role of skin barrier gene mutations and skin barrier impairment in the development of AD and food allergy requires further research. It is likely that there are other hitherto undiscovered skin barrier genes involved. In addition, it is important to study the interplay between genetic and environmental factors, such as water hardness, hygiene practices and the microbiome of the skin.

It is also conceivable that there are other routes to food allergy development than the skin barrier, such as transplacentally or via the gut mucosa, as we know from clinical experience that children can become sensitized to foods without prior development of AD. Building on the hygiene hypothesis and the emerging understanding of the role of bacteria within the host microbiota in the development and maintenance of epithelial cell integrity and tolerance, it has recently been proposed that a lowered bacterial species diversity not only on the skin but also in the gut may lead to inadequate stimulation of immuno-regulatory networks, predisposing to food allergy and AD (Rook, 2009; Hooper et al., 2012). The timing of solid food introduction may be very important in this context, and we are currently testing the hypothesis that the early introduction of allergenic foods at 3 months of age alongside breastfeeding vs exclusive breastfeeding for 6 months reduces the risk of food allergy and AD in the same cohort of children (http://www.eatstudy.co.uk).

We are only beginning to understand how genetic and environmental factors interact at the level of the gut mucosa, the skin and systemically and how this leads to the development of AD and food allergy. The results reported here form a small piece of this jigsaw puzzle and much further work is required. It is hoped that this will not only lead to a better understanding of the patho-aetiology of AD and food allergy but ultimately the development of novel therapeutic interventions and methods of disease prevention.

MATERIALS & METHODS

Our study was approved by the Research Ethics Committee of Guy's & St Thomas' Hospitals Foundation Trust and was conducted according to the Declaration of Helsinki Principles. 619 three-month old exclusively breastfed infants were recruited from the general population in England and Wales through direct advertising between October 2009 and April 2012 for an interventional trial. All children had been born at term (≥37 weeks gestation). Following written informed parental consent and prior to randomization to the study interventions, children were examined for AD, using the UK diagnostic criteria-based photographic protocol of the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Two (Weiland et al., 2004). Disease severity was determined by the Scoring Atopic Dermatitis (SCORAD) index and disease phenotype was recorded ('flexural' (either around the eyes, neck, antecubital and popiteal fossae or ankles) or 'non-flexural') (Kunz et al., 1997). Transepidermal water loss (TEWL) was measured with the Biox Aquaflux® AF200 closed condenser chamber device on the unaffected skin of the volar aspect of the forearm (Farahmand et al., 2009). Participants' parents were advised not to use any skin care

products on the infant's arms for the preceding 24 hours. Measurements were performed in our environmentally controlled Clinical Research Facility (ambient temperature 20 +/− 2 °C, relative room humidity 32-50%), after at least 20 minutes of acclimatization. Measurements were not taken if the child was visibly distressed or crying. In all children we calculated the mean of three separate TEWL measurements. We measured allergic sensitization through skin prick testing, as this has been shown to be more specific as a predictor of clinical food allergy in later life than serum specific IgE (Osborne et al., 2011). Skin prick testing was performed in duplicate to the six commonest allergenic foods in the UK using a combination of whole foods (fresh cow's milk, raw hen's egg white and sesame (tahini) paste) and commercial solutions (peanut, wheat, and cod fish) as well as histamine (10 mg/mL) and normal saline control solutions (all commercial skin prick test solutions Stallergenes, Didcot, UK). A drop of each allergen and both control solutions were placed onto the volar aspect of uninvolved skin of the forearm and pierced vertically using 1 mm ALK lancets. Reactions were recorded after 15 minutes. Two thresholds for determining positive skin prick tests were used in this study, as the predictive value of skin prick tests at such an early age is unknown: any reaction ≥1 mm (primary outcome) and any reaction ≥ 3mm (secondary outcome). Food sensitization was defined as having at least one positive skin prick test to any of the allergens tested. Venous blood samples were screened for the six commonest *FLG* mutations (R501X, 2282del4, R2447X, S3247X, 3673delC, and 3702delG), using the TaqMan allelic discrimination assay (Applied Biosystems, ABI 7900 HT, Foster City, California). We used the Mann Whitney U test and Spearman correlation coefficients to examine median SCORAD and TEWL measurements (both were non-normally distributed) in children with and without AD and with and without *FLG* mutations, using IBM SPSS version 20 (Sun Microsystems, Inc.). Where appropriate, we calculated odds ratio estimates and corresponding 95% confidence intervals, including the association between *FLG* mutation carriage, TEWL, AD, and food sensitization. All odds ratio estimates were adjusted for sex as *a priori* confounder. . We also explored the effect of the degree of TEWL elevation (< 15 vs >15 gram/[m^2×h] – cut off based on the TEWL IQR in unaffected children), AD severity (mild disease-SCORAD<20 vs moderate-to-severe disease SCORAD≥20) and AD phenotype (no AD vs pure flexural vs pure non-flexural vs *both* flexural and non-flexural AD) on food sensitization risk. Since some of the AD variables are strongly correlated (AD phenotype and severity for example) and others potentially on a causal pathway (TEWL is likely to be on the causal pathway between FLG mutation carriage and AD development) we specifically looked for evidence of collinearity influencing the statistical analyses (Schisterman et al., 2009).

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Table 1

The association between *FLG*, TEWL, AD and food sensitization risk (SPT ≥1mm)

	N (%)	Any food sensitization N (%)	Crude OR (95% CI)	P value	OR adjusted for sex and *FLG* (95% CI)	P value
***FLG* mutation**						
No	544 (87.9)	29 (5.3)	1		1	
Yes	75 (12.1)	5 (6.7)	1.27 (0.48-3.38)	0.64	1.21* (0.45-3.24)	0.70
TEWL						
<15 [g/(m^2×h)]	425 (68.7)	15 (3.5)	**1**		**1**	
≥15 [g/(m^2×h)]	194 (31.3)	19 (9.8)	**2.96 (1.47-5.96)**	**0.002**	**3.26* (1.61-6.62)**	**0.001**
AD						
No	465 (75.1)	13 (2.8)	**1**			
Yes	154 (24.9)	21 (13.6)	**5.49 (2.68-11.26)**	**<0.001**	**6.18 (2.94-12.98)**	**<0.001**
AD severity						
No AD	465 (75.1)	13 (2.8)	**1**	**P_{trend} <0.001**	**1**	**P_{trend} <0.001**
SCORAD < 20	132 (21.3)	12 (9.1)	**3.47 (1.55-7.82)**	**0.003**	**3.91 1.70-9.00**	**0.001**
SCORAD ≥ 20	22 (3.6)	9 (40.9)	**24.07 (8.74-66.29)**	**<0.001**	**25.60 (9.03-72.57)**	**<0.001**
AD phenotype						
No AD	465 (75.1)	13 (2.8)	**1**	**P_{trend} <0.001**	1	P_{trend} 0.23
Flexural AD only	44 (7.1)	2 (4.5)	**1.65 (0.36-7.60)**	**0.51**	0.98** (0.20-4.91)	0.98
Non-flexural AD only	36 (5.8)	5 (13.9)	**5.62 (1.88-16.78)**	**0.002**	3.07** (0.92-10.28)	0.26
Both flexural and non-flexural AD	74 (12.0)	14 (19.2)	**8.27 (3.71-18.44)**	**<0.001**	2.55** (0.74-8.83)	0.77

* only adjusted for sex

** adjusted for FLG, sex, and AD severity (SCORAD)

Table 2

The association between *FLG*, TEWL, AD and food sensitization to individual allergens (SPT ≥1mm)

	N (%)	Any food sensitization N (%)	Crude OR (95% CI)	P value	OR adjusted for sex and *FLG* (95% CI)	P value
Egg sensitization						
AD						
No	465 (75.1)	7(1.5)	**1**		**1**	
Yes	154 (24.9)	17 (11.0)	**8.12 (3.30-19.98)**	**<0.001**	**9.48 (3.77-23.83)**	**<0.001**
AD severity						
No AD	465 (75.1)	7 (1.5)	**1**	**P_{trend}<0.001**	**1**	**P_{trend}<0.001**
SCORAD < 20	132 (21.3)	9 (6.8)	**4.78 (1.75-13.11)**	**0.002**	**5.59 (2.00-15.61)**	**0.001**
SCORAD ≥ 20	22 (3.6)	8 (36.4)	**37.39 (11.89-117.53)**	**<0.001**	**41.38 (12.75-134.35)**	**<0.001**
Cow's milk sensitization						
AD						
No	465 (75.1)	3 (0.6)	**1**		**1**	
Yes	154 (24.9)	7 (4.5)	**7.33 (1.87-28.72)**	**0.004**	**9.11 (2.27-36.59)**	**0.002**
AD severity						
No AD	465 (75.1)	3 (0.6)	**1**	**P_{trend}=0.001**	**1**	**P_{trend}<0.001**
SCORAD < 20	132 (21.3)	4 (3.0)	**4.81 (1.06-21.78)**	**0.04**	**6.04 (1.30-28.08)**	**0.02**
SCORAD ≥ 20	22 (3.6)	3 (13.6)	**24.32 (4.60-128.50)**	**<0.001**	**26.55 (4.84-145.61)**	**<0.001**
Peanut sensitization						
AD						
No	465 (75.1)	4 (0.9)	1		**1**	
Yes	154 (24.9)	4 (2.6)	3.07 (0.76-12.44)	0.12	**4.09 (1.00-16.76)**	**0.05**
AD severity						
No AD	465 (75.1)	4 (0.9)	**1**	**P_{trend}=0.001**		**P_{trend}=0.002**
SCORAD < 20	132 (21.3)	2(1.5)	**1.77 (0.32-9.79)**	**0.05**	**2.39 (0.43-13.36)**	**0.32**
SCORAD ≥ 20	22 (3.6)	2 (9.1)	**11.53 (1.99-66.68)**	**0.006**	**13.65 (2.28-81.62)**	**0.004**

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Table 3

Joint modeling of the effect of the skin variables on food sensitization exploring evidence of collinearity

	Food sensitization (SPT≥1mm)				
	FLG **OR (95% CI)**	**TEWL OR (95% CI)**	**AD OR (95% CI)**	**SCORAD OR (95% CI)**	
	Any mutation	**≥15 [g/(m²×h)]**	**Yes**	**<20**	**≥20**
FLG	1.21 (0.45-3.24)	-	-	-	-
FLG, TEWL	0.88 (0.32-2.41)	3.32 (1.61-6.83)‡	-	-	-
FLG, TEWL, AD	0.59 (0.21-1.68)	2.23 (1.04-4.76)*	5.03 (2.33-10.8)§	-	-
FLG, TEWL, SCORAD	0.63 (0.21-1.86)	1.74 (0.77-3.90)	-	3.49 (1.49-8.18)†	19.1 (6.21-58.8)§

All results are adjusted for sex as *a priori* confounder

*p<0.05

†0.01

‡0.001

§0.005