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Filaggrin mutations in relation to skin barrier and atopic dermatitis in early infancy*

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Summary

Background Loss-of-function mutations in the skin barrier gene filaggrin (*FLG*) increase the risk of atopic dermatitis (AD), but their role in skin barrier function, dry skin and eczema in infancy is unclear.

Objectives To determine the role of *FLG* mutations in impaired skin barrier function, dry skin, eczema and AD at 3 months of age and throughout infancy.

Methods *FLG* mutations were analysed in 1836 infants in the Scandinavian population-based PreventADALL study. Transepidermal water loss (TEWL), dry skin, eczema and AD were assessed at 3, 6 and 12 months of age.

Results *FLG* mutations were observed in 166 (9%) infants. At 3 months, carrying *FLG* mutations was not associated with impaired skin barrier function (TEWL > 11·3 g m^{-2} h^{-1}) or dry skin, but was associated with eczema [odds ratio (OR) 2·89, 95% confidence interval (CI) 1·95–4·28; P < 0·001]. At 6 months, mutation carriers had significantly higher TEWL than nonmutation carriers [mean 9·68 (95% CI 8·69–10·68) vs. 8·24 (95% CI 7·97–8·15), P < 0·01], and at 3 and 6 months mutation carriers had an increased risk of dry skin on the trunk (OR 1·87, 95% CI 1·25–2·80; P = 0·002 and OR 2·44, 95% CI 1·51–3·95; P < 0·001) or extensor limb surfaces (OR 1·52, 95% CI 1·04–2·22; P = 0·028 and OR 1·74, 95% CI 1·17–2·57; P = 0·005). *FLG* mutations were associated with eczema and AD in infancy.

Conclusions *FLG* mutations were not associated with impaired skin barrier function or dry skin in general at 3 months of age, but increased the risk for eczema, and for dry skin on the trunk and extensor limb surfaces at 3 and 6 months.

What is already known about this topic?

- Filaggrin (*FLG*) mutations are associated with the development of atopic dermatitis (AD).

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See Appendix.

Conflicts of interest
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Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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- Dry skin is one of the main characteristics of AD and is associated with increased transepidermal water loss (TEWL).
- Impaired skin barrier function measured as increased TEWL has been shown to precede the development of AD.

What does this study add?

- At 3 months of age, *FLG* mutations did not increase the risk of impaired skin barrier function or dry skin in general, but did increase the risk of eczema.
- At 6 months of age, higher TEWL was observed in *FLG* mutation carriers.
- At 3 and 6 months of age, carrying an *FLG* mutation was associated with dry skin on the trunk and extensor limb surfaces, but not with dry skin in general.

What is the translational message?

- Our study highlights the genetic component in skin barrier function, dry skin, eczema and AD in early infancy.

Atopic dermatitis (AD) is a chronic inflammatory disease of the skin and affects 5–20% of children.[1,2] Symptoms of the disease include dry skin, eczematous rash and pruritus, which occur in 60% of infants with AD before 1 year of age.[3]

Filaggrin (*FLG*) is needed for epidermal differentiation, especially for the structure and function of the *stratum corneum*,[4] the outermost layer of the epidermis.[5] Loss-of-function mutations in the *FLG* gene, discovered in 2006 in relation to ichthyosis vulgaris,[6] produce a nonfunctional filaggrin protein and are therefore one of the most prominent risk factors for a dysfunctional skin barrier.[7] Up to 50% of patients with moderate and severe AD are carriers of at least one *FLG* mutation,[8] and mutation carriers more often show an earlier onset of the disease.[9] In a European population, five different mutations in the *FLG* gene account for 96% of the total risk alleles, while 86% of the total risk alleles include 16 different mutations in the Asian population.[10]

Carrying an *FLG* mutation increases the risk of AD;[11–14] however, associations with AD are less clear in infants younger than 1 year of age. Dry skin is a cardinal sign of AD observed in most children with the disease,[15,16] and is associated with increased transepidermal water loss (TEWL) across the *stratum corneum*.[16,17] The impaired skin barrier function measured as TEWL has been found to precede the development of AD.[18] Limited information is available for the outcomes of impaired skin barrier function, dry skin, eczema and AD in a combined analysis from a large cohort before 1 year of age.

Thus, we aimed primarily to determine the role of *FLG* mutations for impaired skin barrier function, dry skin, eczema and AD at 3 months of age in a prospective Scandinavian cohort. Our secondary aim was to explore whether *FLG* mutations are associated with skin barrier function, dry skin, eczema and AD in the first year of life.

Patients and methods

Study design

The present study includes all infants in the Scandinavian multicentre, prospective birth cohort study Preventing Atopic Dermatitis and Allergies in Children (PreventADALL) with available DNA for genotyping who attended at least one clinical investigation at 3, 6 or 12 months of age. Detailed information on the study design and baseline characteristics of the PreventADALL study are published elsewhere.[19] Briefly, infants were recruited antenatally in connection with the national routine 18-week ultrasound examination in three hospital areas in Oslo and Østfold (Norway) and in Stockholm (Sweden). The inclusion criteria were singleton or twin pregnancies at 16–22 weeks gestational age, and sufficient Scandinavian language skills to comply with the study. The exclusion criteria were severe fetal and/or maternal disease, and infants born prior to 35 weeks gestational age. Information on baseline characteristics was collected using electronic questionnaires at 18 and 34 weeks gestational age. Clinical follow-up visits for the infants took place at 3, 6 and 12 months of age.

The study was approved by the Regional Committee for Medical and Health Research Ethics in Norway (2014/518) and the Swedish Ethical Review Authority (2014/2242-32/4) and is registered at ClinicalTrials.gov (NCT02449850). The mothers signed the consent form during pregnancy at the primary enrolment and signed again, together with the coparent, after birth at the enrolment of the child.

Study population

Our study sample of 1836 infants was similar to the sample of infants enrolled in the PreventADALL study without

available DNA with respect to baseline characteristics, except for mode of delivery and parental education (Table S1; see Supporting Information).

Methods

DNA was isolated from blood and genotyped using the TaqMan-based allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). Further details are provided in File S1 (see Supporting Information). If the genotyping analysis showed the result 'undetermined', the values were labelled as missing values. We defined *FLG* mutations ('mutation yes') as being a carrier of any of the mutations R501X, 2282del4 and R2447X of the *FLG* gene, the most common loss-of-function mutations in the European population.

Skin assessments were performed at each visit (3, 6 and 12 months of age) by study personnel who were specifically trained by dermatologists in evaluating infant skin, including dry skin without visible signs of inflammation, eczema, and the use of diagnostic criteria for AD.[20] Yearly workshops were organized in the three study centres to ensure interobserver agreement. Parents were asked not to bathe the infant or apply any emollients for at least 24 h prior to the visit.

Skin barrier function was measured by TEWL (g m^{-2} h^{-1}) at 3, 6 and 12 months in triplicate. We applied the open chamber DermaLab USB (Cortex, Hadsund, Denmark) at room temperature between 20°C and 25°C, in line with international recommendations,[21] accepting humidity within the range 5·7–80·6% with a mean of 30%, in line with previous studies in our laboratory.[18,22] Approximately 15 mins after acclimatization, the measurements were performed on the left upper lateral arm of calm infants. The surrounding temperature and the humidity levels were noted and the windows and doors were kept closed during the measurement. The TEWL values are repeated as the mean of three successfully performed measurements.

Outcomes and definitions

Skin barrier function was measured by assessing mean TEWL (g m^{-2} h^{-1}) at group level at 3, 6 and 12 months. Impaired skin barrier function was defined as a high TEWL > 11·3 g m^{-2} h^{-1} based on a previous report for 3-month-old infants in the same cohort.[23]

Health personnel were trained to examine the skin by visual inspection and palpation. Dry skin was defined as the presence of scaling and roughness in at least one of the recorded 11 predefined anatomical skin areas at 3, 6 and 12 months,[24] excluding infants with the concurrent presence of eczema and AD at the respective timepoints.

The presence of eczematous skin lesions was verified by a physician, clinically excluding common differential diagnosis to AD, e.g. seborrhoeic and contact dermatitis.

AD was diagnosed based on the UK Working Party (UKWP)[25–27] criteria at either 3, 6 or 12 months of age and/or Hanifin and Rajka criteria[28] at 12 months, while 'ever' AD was defined as fulfilling the UKWP and/or Hanifin and Rajka criteria by 12 months of age (Table S2; see Supporting Information).

Statistical analysis

Continuous variables are presented as means, SDs and minimum–maximum (min–max); categorical variables are presented as numbers and percentages.

To determine the role of *FLG* mutations in impaired skin barrier function, dry skin, eczema and AD at 3 months, we used regression models with *FLG* mutations as the independent variable in univariate analyses followed by multivariable analyses adjusting for sex. Results for binary outcomes are reported as odds ratios (ORs) with 95% confidence intervals (CIs).

The TEWL values for mutation carriers vs. nonmutation carriers were compared using a two-tailed t-test. For analyses of the associations between *FLG* and categorical outcomes (dry skin, dry skin locations, eczema and AD), we used χ^2-tests. All analyses were conducted using IBM SPSS statistics version 25 (Armonk, NY, USA). The significance level was set to 5%, not adjusting for multiplicity.

Results

The background characteristics of the 1836 infants (968 boys) are given in Table 1. The prevalence of carrying at least one of the three mutations (R501X, 2282del4, R2447X) was 9% (n = 166), as shown in Figure 1.

At 3 months of age, neither impaired skin barrier function nor dry skin in general was associated with *FLG* mutations in the univariate analysis, or in the multivariable analysis when adjusting for sex. The risk of eczema was higher in infants with *FLG* mutations in the univariate analysis (OR 2·91, 95% CI 1·97–4·31; P < 0·001) and also after adjusting for covariates (OR 2·89, 95% CI 1·95–4·28; P < 0·001). The multivariate analysis also indicated a higher risk for AD among *FLG* mutation carriers (OR 2·32, 95% CI 0·77–7·91; P = 0·134) (Table 2).

The mean TEWL (g m^{-2} h^{-1}) was higher among infants with *FLG* mutations compared with nonmutation carriers [9·68 (95% CI 8·69–10·68) vs. 8·24 (95% CI 7·97–8·15)] at 6 months of age, but not at the other timepoints (Figure 2 and Table S3; see Supporting Information).

Dry skin in general, in addition to dry skin on the infant cheeks, was not significantly associated with *FLG* mutations at 3, 6 or 12 months of age, as shown in Table 3. However, dry skin located on the trunk and extensor limb surfaces was significantly associated with *FLG* mutations at age 3 months (OR 1·87, 95% CI 1·25–2·80; P = 0·002 and OR 2·44, 95% CI 1·51–3·95; P < 0·001) and at age 6 months (OR 1·52, 95% CI 1·04–2·22; P = 0·028 and OR 1·74, 95% CI 1·17–2·57; P = 0·005), respectively.

Carrying *FLG* mutations was significantly associated with the presence of eczema in infants at all three timepoints, and for AD from 6 months of age (Table 3).

Table 1 Baseline characteristics of the 1836 infants with filaggrin (*FLG*) genotyping are shown, based on the presence (n = 166) or absence (n = 1670) of at least one *FLG* mutation

	FLG mutation, no (n = 1670)	*FLG* mutation, yes (n = 166)	P-values[a]
Sex of child	n = 1670	n = 166	
Girls	798 (47·8)	70 (42·2)	0·167
Boys	872 (52·2)	96 (57·8)	0·167
Age of mother at 18 weeks enrolment	n = 1670	n = 166	
Mean age, years (SD, min–max)	32·4 (4·1, 21·0–48·0)	32·5 (4·1, 20·0–47·0)	0·803
Age of father at 18 weeks enrolment	n = 1432	n = 149	
Mean age, years (SD, min–max)	34·7 (5·4, 21·0–65·0)	34·4 (5·1, 23·0–51·0)	0·443
Mother educational level	n = 1517	n = 156	
Preliminary only	11 (0·7)	1 (0·6)	0·906
High school only	143 (9·4)	14 (9·0)	0·854
Higher education < 4 years	483 (31·8)	43 (27·6)	0·273
Higher education ≥ 4 years	878 (57·9)	98 (62·8)	0·233
Other	2 (0·1)	0 (0·0)	0·650
Father educational level	n = 1469	n = 154	
Preliminary only	20 (1·4)	0 (0·0)	0·145
High school only	260 (17·7)	21 (13·6)	0·205
Higher education < 4 years	448 (30·5)	42 (27·3)	0·407
Higher education ≥ 4 years	729 (49·6)	91 (59·1)	**0·025**
Other	12 (0·8)	0 (0·0)	0·260
Mother Nordic origin	n = 1523	n = 157	
Norway	1033 (67·8)	118 (75·2)	0·060
Sweden	326 (21·4)	26 (16·6)	0·156
Other Nordic countries	18 (1·2)	3 (1·9)	0·434
Rest of the world	146 (9·6)	10 (6·4)	0·186
Father Nordic origin	n = 1490	n = 155	
Norway	996 (66·8)	118 (76·1)	**0·019**
Sweden	324 (21·7)	28 (18·1)	0·288
Other Nordic countries	14 (0·9)	2 (1·3)	0·672
Rest of the world	156 (10·5)	7 (4·5)	**0·018**
Maternal prepregnancy BMI	n = 1646	n = 162	
Mean BMI (SD, min–max)	24·7 (3·5, 17·22–42·50)	24·8 (3·8, 18·47–48·16)	0·712
Mode of delivery	n = 1668	n = 166	
Vaginal delivery	1425 (85·4)	142 (85·5)	0·969
Caesarean section	243 (14·6)	24 (14·5)	0·969
Number of previous deliveries	n = 1668	n = 166	
0	1001 (60·0)	88 (53·0)	0·080
1	524 (31·4)	61 (36·7)	0·160
≥ 2	143 (8·6)	17 (10·2)	0·468
Birth weight	n = 1667	n = 165	
Mean weight (g) (SD, min–max)	3589·4 (462·8, 2005·0–5632·0)	3622·0 (494·6, 1933·0–4940·0)	0·390
Length of baby at birth	n = 1598	n = 161	
Mean length (cm) (SD, min–max)	50·6 (2·1, 34·0–61·0)	50·7 (2·0, 44·0–56·0)	0·554
Maternal doctor-diagnosed AD	n = 1523	n = 157	**0·022**
	291 (19·1)	42 (26·8)	
Paternal doctor-diagnosed AD	n = 1528	n = 153	0·444
	157 (10·3)	20 (13·1)	

BMI, body mass index. [a]P-values are based on independent samples *t*-tests and χ^2-tests for differences in distributions between infants without and with *FLG* mutations. Data are provided as n (%) unless otherwise stated. Bold indicates P-values < 0.05.

Discussion

We did not find evidence that infants who carry at least one *FLG* loss-of-function mutation have an increased risk of impaired skin barrier function or dry skin in general at 3 months of age; however, we found that the risk of eczema was three times higher in mutation carriers than for infants who did not carry a mutation. At 6 months of age, infants with *FLG* mutations had higher TEWL; however, there was no association between *FLG* mutations and dry skin in general or dry skin on the cheeks at any timepoint. Nevertheless, carrying an *FLG* mutation increased the risk for dry skin on the trunk and extensor limb surfaces at 3 and 6 months of age, and for eczema at all three timepoints. Infants with *FLG*

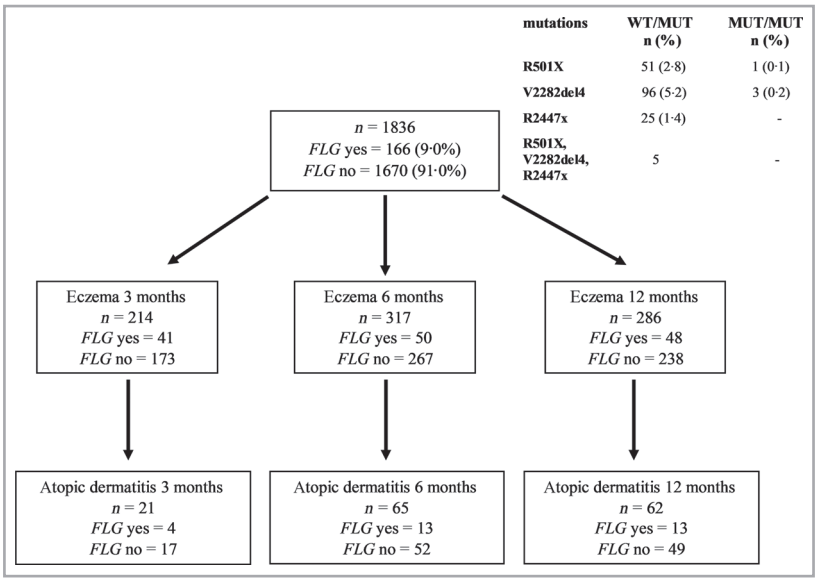

Figure 1 Among the 1836 infants in the PreventADALL study, the number of infants with eczema and atopic dermatitis, respectively at 3, 6 and 12 months of age is shown, based on the presence or absence of carrying at least one of three common filaggrin (*FLG*) mutations. The distribution of *FLG* mutations by heterozygous mutations wildtype/mutation (WT/MUT) and homozygous mutations (MUT/MUT) is displayed in the upper right corner.

Table 2 In a general population of 1836 infants, the risk of impaired skin barrier function, dry skin, eczema and atopic dermatitis (AD) at 3 months of age by carrying filaggrin (*FLG*) mutations is shown in univariate (crude) and multivariate logistic regression analysis adjusted for infant sex

Outcome	Crude model OR	95% CI	P-values	Adjusted model OR[a]	95% CI	P-values
Impaired skin barrier function (n = 228)	1·44	0·91–2·27	0·119	1·42	0·90–2·34	0·135
Dry skin (n = 744)	1·34	0·96–1·87	0·091	1·34	0·96–1·87	0·089
Eczema (n = 214)	2·91	1·97–4·31	**< 0·001**	2·89	1·95–4·28	**< 0·001**
AD (n = 21)	2·41	0·80–7·24	0·119	2·32	0·77–7·91	0·134

OR, odds ratio; CI, confidence interval. [a]Adjusted for infant sex. Bold indicates P-values < 0.05.

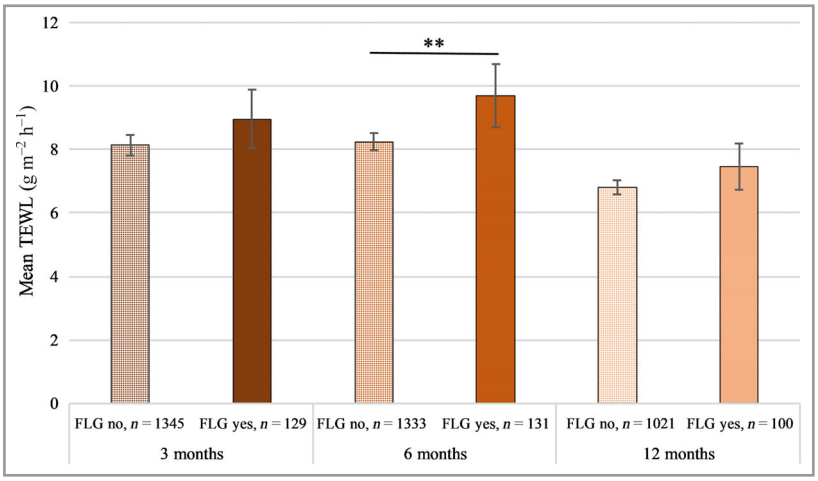

Figure 2 The mean transepidermal water loss (TEWL) is shown at 3, 6 and 12 months of age for 1836 infants, based on the presence or absence of at least one filaggrin (*FLG*) loss-of-function mutation (*FLG* yes). The mean TEWL was significantly higher in mutation carriers compared with nonmutation carriers at 6 months of age.

Table 3 Clinical characteristics at 3, 6 and 12 months of age in infants with and without filaggrin (*FLG*) mutations. The unadjusted crude odds ratio (OR) with 95% confidence intervals (CIs) are provided for each characteristic, using the absence of the characteristic as reference

	FLG mutations No, n = 1670	*FLG* mutations Yes, n = 166	P-values[a]	OR (95% CI)
Dry skin				
3 months	667 (44·1)	77 (51·3)	0·090	1·34 (0·96–1·87)
6 months	603 (41·6)	64 (45·7)	0·345	1·18 (0·84–1·68)
12 months	597 (43·2)	54 (40·9)	0·617	0·91 (0·63–1·31)
Dry skin cheeks				
3 months	401 (26·7)	38 (26·4)	0·933	0·98 (0·67–1·45)
6 months	432 (29·9)	41 (29·3)	0·876	0·97 (0·66–1·42)
12 months	427 (31·1)	35 (26·5)	0·280	0·80 (0·54–1·20)
Dry skin truncus				
3 months	218 (14·6)	36 (24·2)	**0·002**	1·87 (1·25–2·80)
6 months	113 (7·9)	24 (17·3)	**< 0·001**	2·44 (1·51–3·95)
12 months	128 (9·4)	17 (13·0)	0·181	1·44 (0·84–2·48)
Dry skin extensor limb surfaces				
3 months	321 (21·4)	43 (29·3)	**0·028**	1·52 (1·04–2·22)
6 months	262 (18·2)	39 (27·9)	**0·005**	1·74 (1·17–2·57)
12 months	304 (22·1)	34 (25·8)	0·335	1·22 (0·81–1·84)
Eczema				
3 months	173 (11·4)	41 (27·3)	**< 0·001**	2·91 (1·97–4·31)
6 months	267 (18·4)	50 (35·7)	**< 0·001**	2·46 (1·70–3·57)
12 months	238 (17·2)	48 (36·4)	**< 0·001**	2·75 (1·88–4·02)
Atopic dermatitis diagnosed				
3 months	17 (1·1)	4 (2·7)	0·108	2·41 (0·79–7·24)
6 months	52 (3·6)	13 (9·3)	**0·001**	2·74 (1·46–5·18)
12 months	49 (3·5)	13 (9·8)	**< 0·001**	2·97 (1·57–5·63)
Ever (0–12 months)	131 (8·5)	39 (25·3)	**< 0·001**	3·66 (2·44–5·48)

[a]P-values were based on χ^2-tests. Data are provided as n (%) unless otherwise stated. Bold indicates P-values < 0.05.

mutations also had a 2·5-fold to threefold higher risk of being diagnosed with AD at 6 or 12 months of age, and at any point up to 1 year of age.

The prevalence of *FLG* mutation carriers (9%) for the investigated mutations in the present study is comparable with other European cohorts,[29] whereas different mutations are more prevalent in other populations.[10,30] However, independent of the exact mutation, associations between *FLG* and the development of AD and eczema have been found in several different populations.[10,29–31]

The current and previous studies have shown that *FLG* mutations are associated with skin barrier function,[32] dry skin,[31] eczema[32,33] and AD.[31] This highlights the important role of filaggrin for correct skin differentiation. In the stratum corneum, filaggrin is degraded and makes the bases for the natural moisturizing factor (NMF), which is important for correct epidermal barrier function as part of the chemical barrier of the skin.[34] However, owing to loss-of-function mutations such as those investigated in this study, a functional filaggrin protein cannot be produced, resulting in decreased NMF levels,[35] in addition to a dysregulation in keratinocyte differentiation[36] and damage to tight junctions.[37] The *FLG* gene is located in the epidermal differentiation complex, which includes a number of genes that encode several major proteins that are essential for correct epidermal differentiation.[38]

The lack of association between *FLG* mutations and impaired skin barrier function (high TEWL), in addition to dry skin in general at 3 months of age in our study, is in contrast to the observed associations with both outcomes at the same age reported by Flohr *et al.*[32] Although both studies recruited participants from the general population, there are important differences between the two study populations; our study included 1836 infants, of whom 9% had an *FLG* mutation and 12% had clinical eczema at 3 months, whereas the UK study included 88 infants, of whom 17% had *FLG* mutations and 33% had clinical eczema. Therefore, the identification of significant associations between *FLG* mutations and TEWL, in addition to observed dry skin, may be less likely across a large general population compared with a smaller cohort enriched by clinically manifested disease. Alternatively, it is possible that *FLG* mutations may have less impact on the skin barrier in the first months of life, which is supported by our findings of significant associations with TEWL first observed at 6 months of age. The lack of association between *FLG* mutations and dry skin at 3 months may indicate that dry skin at this age represents heterogeneous phenotypes, only partly overlapping with eczema and AD. As most infants with eczema also had dry skin, our analyses relating to *FLG* mutations and dry skin excluded infants with eczema. Our finding that carrying *FLG* mutations increased the risk of eczema at 3 months is in line

with that reported by Flohr *et al.*[32] In our study, *FLG* mutation carriers also had an increased risk for AD at 3 months of age. However, as only 21 infants fulfilled the UKWP criteria for AD, our findings need further confirmation in independent studies.

Our finding that skin barrier function (increased TEWL) is associated with *FLG* mutations at 6 months is supported by Berents *et al.*[22] who observed significantly higher TEWL among 167 infants at 6 months of age in the 8% of infants carrying *FLG* mutations. Furthermore, increased TEWL has been shown in mutation carriers among Korean children aged 10–14 years.[30]

The lack of association in our study between *FLG* mutations and dry skin in general in infancy is contrary to previous findings, which found that *FLG* mutations increased the risk of dry skin at 3 months[32] and at 7–8 years[31,39] in population-based studies. Moreover, we did not find an association between dry skin on the cheeks and mutation carriers, which may in part be explained by a study by McAleer *et al.*,[40] which examined NMF levels (the degradation product of filaggrin) in children aged up to 6 years, excluding those with a history suggestive of AD or another inflammatory skin disease. The authors reported low NMF levels in infants after birth, while NMF levels in the cheeks increased more slowly compared with the levels found in the tip of the nose and elbow flexure. The authors also found that steady-state NMF levels on the cheeks are not reached until school age.[40] Thus, *FLG* may be differently regulated in the cheeks compared with other body areas, which could explain the lack of association between *FLG* mutations and dry skin on the cheeks in infancy in our study. Furthermore, environmental factors may have a greater impact on dry skin on the cheeks compared with other body areas that are less exposed.

This is supported by our finding that dry skin located on the trunk and extensor limb surfaces was associated with *FLG* mutations at 3 and 6 months of age. We are not aware of other studies that report the role of *FLG* mutations in the distribution of dry skin in early childhood. However, Thyssen *et al.*[41] reported an increased risk of fissures on the hands and/or fingers in adults with an *FLG* mutation (R501X, 2282del4) who did not have a diagnosis of AD. Our data indicate that *FLG* mutations have a differential effect on the development of dry skin restricted to specific locations, but further studies are needed to validate these findings.

The increased risk of eczema in infants with *FLG* mutations throughout infancy in our study is supported by a study involving infants at 3 months of age[32] and another small study involving 17 infants at 6 months of age with *FLG* gene expression assessed in cord blood.[42]

Moreover, infants with *FLG* mutations were at increased risk of an AD diagnosis within the first year of life, in line with previous reports.[11–14] Our results even indicate an association between *FLG* and AD at 3 months of age. However, diagnosing AD at this early age may be challenging owing to the infant's inability to scratch themselves intentionally. We have previously reported that the current diagnostic criteria for diagnosing AD may therefore be of limited value in early infancy,[43] and some of these infants presenting with eczema at 3 months of age will later fulfil the diagnostic criteria for AD. Our study adds to previous knowledge that an association between *FLG* and eczema can be observed before 1 year of age and showed an association between *FLG* and an AD diagnosis at 6 and 12 months of age.

Our study has several strengths. The detailed characterization of infants at 3, 6 and 12 months of age, by diligently trained study personnel, enabled assessments of the genetic contribution to the developing skin from an early age at which environmental exposure was limited. The large number of participants from a general population, renders the results largely generalizable, with *FLG* mutations mainly as expected in relevant populations. The standard operating procedure was the same for all centres, and the study personnel were trained together to achieve a high level of consistency between the study centres. A high participation rate was achieved at all three timepoints (89% at 3 months, 84% at 6 months, 80% at 12 months). A potential limitation of the study could be the randomization of infants to skincare interventions (or not). However, we previously demonstrated that the skin intervention was not effective in reducing AD or eczema by 12 months of age,[20] and investigators were blinded to the intervention randomization of the participants. Therefore, we believe that a potential impact of the interventions would be minimal for the present results. A further limitation could be that the study missed out non-European mutations, as the infants were genotyped only for the three most frequent mutations in the European population (R501X, 2282del4 and R2447X). Another limitation of our results could be the use of an open-air chamber to measure TEWL without stringent ambient humidity control, as the humidity ranged between 5·7% and 80·6% in our study. However, we found no significant association between ambient humidity and TEWL (data not shown) in the present study, or in previous studies using the same equipment and, in Oslo, using the same facilities.[22]

The participants in our study population had a higher level of education and may therefore be more interested in taking part in the study. The origins of the parents can imply different genetic variations, which could potentially affect the generalizability of our results and the results of other similar clinical studies. However, the prevalence of *FLG* mutations (9%) in our study is comparable with that found in other European populations.

In this large general population study, 9% of participants were *FLG* mutation carriers, and we found no evidence of association of *FLG* mutations with impaired skin barrier function or with dry skin in general at 3 months; however, we found that *FLG* mutations increased the risk of eczema approximately threefold. *FLG* mutations were further associated with dry skin on the trunk and extensor limb surfaces at 3 and 6 months of age. An association between *FLG* mutations and eczema was also present throughout the first year of life, in addition to an association between *FLG* mutations and AD by age 1 year. In conclusion, our study highlights the genetic component for dry skin, skin barrier function, eczema and AD in infants.

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Appendix

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1 Baseline characteristics of the study population of 1836 infants with filaggrin (*FLG*) genotyping compared with 559 infants who had no genotyping (not included).
Table S2 UK Working Party criteria, modified for use in infancy.
Table S3 Mean transepidermal water loss (g m^{-2} h^{-1}) is given, comparing individuals with and without filaggrin (*FLG*) mutations.
File S1 Supplementary methods.
Powerpoint S1 Journal Club Slide Set.