

Filaggrin loss-of-function mutations are not associated with atopic dermatitis that develops in late childhood or adulthood

H. Rupnik,^{1,3} M. Rijavec² and P. Korošec²

¹Department of Dermatovenereology, University Medical Centre Ljubljana, Ljubljana, Slovenia

²University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia

³Dermatology Centre Arsderma, Ljubljana, Slovenia

Summary

Correspondence

Peter Korošec.

E-mail: peter.korosec@klinika-golnik.si

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Background The influence of filaggrin gene (FLG) mutations on early- vs. late-onset development of atopic dermatitis (AD), allergic contact dermatitis (ACD) and chronic irritant contact dermatitis (CICD) is not completely understood.

Objectives To assess the association between FLG mutations and development of AD, ACD and CICD.

Methods This study assessed 241 patients with AD. AD developed during infancy in 85 patients, during childhood in 79 patients (32 early and 47 late) and during adulthood in 77 patients. We also included 100 patients with ACD and 44 with CICD, as well as 164 healthy controls. Four prevalent FLG loss-of-function mutations were genotyped (R501X, 2282del4, R2447X and S3247X).

Results The 2282del4 mutation was significantly associated with a greater risk of AD in the entire group [odds ratio (OR) 4.33, 95% confidence interval (CI) 1.26–14.96]. However, the 2282del4 mutation was associated only with AD that developed during infancy or in early childhood (≤ 8 years: OR 20.91, 95% CI 2.73–159.9), not with AD development in late childhood or adulthood (> 8 or > 18 years), or ACD or CICD. Similar associations were also observed for the combined 2282del4 or R501X genotype. Carriers of FLG mutations also experienced a longer duration of AD and required hospitalization more often.

Conclusions FLG mutations are associated with only the early onset of AD, not late onset. Other factors should receive attention in patients with late-onset AD.

What's already known about this topic?

- Filaggrin gene (FLG) loss-of-function mutations are a major risk factor for atopic dermatitis (AD).
- It is not clear whether FLG mutations are also important for late childhood or adult onset of AD, which develops in up to 20% of patients.

What does this study add?

- The development of AD during late childhood or adulthood is not associated with FLG loss-of-function mutations.
- Other susceptibility genes and/or other types of dysregulation are likely to be involved in the development of late-onset AD.

Filaggrin is one of the most important epidermal structural proteins involved in epidermal barrier homeostasis. Filaggrin is encoded by the gene FLG, which is located within the epidermal differentiation complex on chromosome 1q21.^{1,2} FLG

null mutations result in an impaired epidermal barrier, thereby permitting increased penetration of allergens, haptens and irritants.¹ More than 40 FLG mutations have been identified.² The most common mutations in European studies

include R501X and 2282del4,³ and the carrier frequencies differ substantially, from 1.4% in an Italian population⁴ to 56% in an Irish population.⁵ One of the first association studies⁵ revealed that FLG null mutations predispose individuals to atopic dermatitis (AD), and numerous studies later confirmed this observation, with odds ratios (ORs) ranging from approximately 2 to 7.^{6–11} However, all of these studies focused on patients with early-onset AD; thus, the role of FLG mutations in patients with late-onset AD development is not completely understood. Importantly, although AD develops during infancy or early childhood in the majority of patients, it develops in adulthood in up to 20% of patients.^{12–14}

In addition to AD, other skin diseases are also associated with skin barrier abnormalities, including allergic contact dermatitis (ACD) and chronic irritant contact dermatitis (CICD). The positive association between FLG loss-of-function mutations and ACD has been noted only in subgroups of patients with nickel allergy^{15,16} and not in unselected patients with ACD.^{15–19} Some recent studies have indicated an increased susceptibility of FLG mutation carriers to CICD.^{20–22}

The primary objective of this study was to determine whether an association exists between AD development in infancy, childhood or adulthood and FLG loss-of-function mutations. The study sample consisted of patients who developed AD during infancy, patients who developed AD during childhood, and patients who developed AD during adulthood. Furthermore, the prevalence of FLG mutations was also analysed according to the duration of AD, the need for hospitalization, and concomitant allergic diseases. In addition, we sought to assess the association between FLG mutations and ACD and CICD.

Materials and methods

Study populations

Patients were enrolled prospectively between March 2009 and June 2012 at the Department of Dermatovenereology, Univer-

sity Medical Centre Ljubljana. The study was designed such that it consisted of one-third patients who developed AD during infancy, one-third patients who developed AD during childhood (approximately half during early childhood and half during late childhood), and one-third patients who developed AD during adulthood. Thus, 85 patients (35%) developed AD by age 2 years, 79 patients (33%) developed AD at age 2–18 years (32 patients at 2–8 years and 47 patients at 8–18 years) and 77 patients (32%) developed AD at > 18 years. Altogether, 241 patients with AD were included in this study. We also included 100 patients with ACD, 44 patients with CICD and 164 control subjects. The detailed demographic and clinical characteristics of all patients and control subjects are presented in Table 1.

The study was approved by the Slovenian National Medical Ethics Committee. Written informed consent was obtained from all participants or their parents prior to enrolment in the study.

The diagnosis of AD, ACD or CICD was made by a dermatologist at the clinical research unit who was experienced in allergic skin diseases in line with established guidelines.²³ The diagnosis of AD was made clinically according to the Hanifin and Rajka criteria (three of the four major criteria in addition to three of 21 minor criteria).²³ The diagnosis of ACD was based on a clinical examination and a history of dermatitis occurring a few hours or days after specific allergen exposure, or persistent dermatitis in the case of repeated exposure and a positive patch test result. The diagnosis of CICD was made when irritant contact dermatitis persisted for at least 3 months. A positive patch test result was an exclusion criterion. The control group included healthy individuals without any allergic disorder or ichthyosis vulgaris. A family history of atopy was not an exclusion criterion. The control subjects were older than the patients to reduce the probability that they would have developed the skin diseases relevant to this study.

During the clinical examination, relevant clinical data were obtained from all patients, including age at onset, duration of disease, history of hospitalizations, personal and family history

Table 1 Demographic and clinical characteristics of the participants

	AD	ACD	CICD	Controls
Number of patients	241	100	44	164
Age (years), mean $\pm$ SD	23.5 $\pm$ 12.2	42.1 $\pm$ 16.5	40.3 $\pm$ 14.9	41.7 $\pm$ 20.2
Male sex, n (%)	75 (31.1)	29 (29)	15 (34)	103 (62.8)
Disease onset (years), mean $\pm$ SD	12.5 $\pm$ 13.3	38.0 $\pm$ 17.0	33.8 $\pm$ 15.3	
Age of disease onset, n (%)				
Infancy (0–2 years)	85 (35.3)			
Early childhood (2–5 years)	15 (6.2)			
Early to mid childhood (5–8 years)	17 (7.1)			
Late childhood (8–18 years)	47 (19.5)			
Adulthood (> 18 years)	77 (32.0)			
Disease duration (years), mean $\pm$ SD	10.9 $\pm$ 10.2	4.3 $\pm$ 6.9	6.9 $\pm$ 7.1	
Hospitalization, n (%)	83 (34.4)	13 (13)	2 (5)	
Asthma, n (%)	57 (23.7)	11 (11)	8 (18)	
Allergic rhinoconjunctivitis, n (%)	68 (28.2)	14 (14)	5 (11)	

AD, atopic dermatitis; ACD, allergic contact dermatitis; CICD, chronic irritant contact dermatitis.

of asthma, allergic rhinitis, food allergy, drug allergy, urticaria and angio-oedema. Data regarding IgE sensitization and patch test results were obtained from the patients' medical charts. Blood samples of all subjects were collected at the time of inclusion in the study.

DNA isolation and genotyping of FLG mutations

Genomic DNA was extracted from ethylenediaminetetraacetic acid-containing whole blood samples using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

FLG mutations R501X (rs61816761), 2282del4 (rs41370446), R2447X (rs138726443) and S3247X (rs150597413) were determined using a Taqman-based allelic discrimination assay as reported previously.^{5,24,25} All experiments used appropriate controls (wild-type, heterozygous and homozygous variants for all mutations). Genotyping was performed on an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA, U.S.A.) with SDS v2.0.5 software.

Statistical analyses

Possible deviation from the Hardy–Weinberg equilibrium was tested using a χ^2 -test. Data distribution was evaluated using the D'Agostino and Pearson test. Parametric statistics (paired and unpaired t-tests) were used for normally distributed data, and nonparametric statistics (the Mann–Whitney, Wilcoxon and Kruskal–Wallis tests) were used if the distribution deviated from normal. The two-sided Fisher's exact test was used to calculate the significance of the differences in FLG mutation

distributions. We used GraphPad Prism software (version 5.0 for Windows; GraphPad Software, San Diego, CA, U.S.A.). A P-value < 0.05 was considered statistically significant. Power analysis was performed using the genetic power calculator available at <http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html>.²⁶ The power was estimated to detect a dominant effect, considering a disease prevalence of 15%, using the FLG mutation allele frequency, and type I error rate (α) of < 0.05.

Results

FLG loss-of-function mutation analysis

In the AD group, 11% (3.3%, 7.5%, 0.4%, and 0% for R501X, 2282del4, R2447X and S3247X, respectively) of patients carried at least one FLG loss-of-function mutation, compared with 6% (4%, 1%, 0% and 1%, respectively) in the ACD group, 5% (5%, 0%, 0% and 0%, respectively) in the CICD group and 5.5% (1.8%, 1.8%, 1.2% and 0.6%, respectively) in the healthy control group (Table 2). We compared the AD, ACD and CICD groups with the control group; the only significant association was noted for the AD group and the 2282del4 mutation [$P = 0.012$; OR 4.33, 95% confidence interval (CI) 1.26–14.96]. In contrast, none of the analysed FLG mutations were associated with ACD or CICD. The combination of patients with R501X and patients with 2282del4 also exhibited a significant association with AD ($P = 0.009$; OR 3.18, 95% CI 1.28–7.92). Among patients with AD, one compound heterozygote (R501X/2282del4) was identified, whereas no homozygotes were identified in any of the subjects tested.

Table 2 Prevalence of FLG variants according to diagnosis

FLG variant	AD (n = 241)	ACD (n = 100)	CICD (n = 44)	Controls (n = 164)
R501X				
n (%)	8 (3.3)	4 (4)	2 (5)	3 (1.8)
OR (95% CI)	1.84 (0.48–7.05)	2.24 (0.49–10.21)	2.56 (0.41–15.80)	
P-value	0.54	0.43	0.29	
2282del4				
n (%)	18 (7.5)	1 (1)	0	3 (1.8)
OR (95% CI)	4.33 (1.26–14.96)	0.54 (0.06–5.29)	0.52 (0.03–10.23)	
P-value	0.012	1	1	
R2447X				
n (%)	1 (0.4)	0	0	2 (1.2)
OR (95% CI)	0.34 (0.03–3.76)	0.32 (0.02–6.81)	0.73 (0.03–15.50)	
P-value	0.57	0.53	1	
S3247X				
n (%)	0	1 (1)	0	1 (0.6)
OR (95% CI)	0.22 (0.01–5.56)	1.63 (0.10–26.37)	1.23 (0.05–30.61)	
P-value	0.40	1	1	
R501X or 2282del4				
n (%)	26 (10.8)	5 (5)	2 (5)	6 (3.7)
OR (95% CI)	3.18 (1.28–7.92)	1.39 (0.41–4.67)	1.25 (0.24–6.44)	
P-value	0.009	0.75	0.68	

AD, atopic dermatitis; ACD, allergic contact dermatitis; CICD, chronic irritant contact dermatitis; OR, odds ratio; CI, confidence interval.

***FLG* loss-of-function mutation analysis in patients with atopic dermatitis according to different disease onset periods**

Next, we analysed patients with AD according to the development of the disease. In our study, 85 patients (35%) experienced early-onset AD (≤ 2 years), 79 patients (33%) experienced childhood-onset AD (2–18 years) and 77 patients (32%) experienced adult-onset AD (> 18 years). We observed a considerably higher frequency of the 2282del4 mutation or the combined R501X or 2282del4 mutation in patients with an early onset of disease (≤ 2 years) than in patients who developed AD after 2 years of age ($P = 0.008$ for both 2282del4 and R501X or 2282del4) or in healthy controls ($P < 0.001$ for both 2282del4 and R501X or 2282del4; Table 3). Furthermore, the strong association between the 2282del4 mutation or the combined R501X/2282del4 mutation was evident throughout all of early childhood, and the peak difference was noted in patients stratified as having an onset of AD at ≤ 8 years compared with patients who developed AD at > 8 years old ($P < 0.001$ for both groups) or healthy controls ($P < 0.001$ for both groups; Table 3 and Figure 1). Interestingly, if patients were stratified in ranked subgroups, the highest 2282del4 mutation frequency (20%) was evident in the 3–5-year-old subgroup. In contrast, in patients experiencing AD onset during late childhood or as an adult (onset of > 8 to ≤ 18 or > 18 years), the frequencies of the 2282del4 mutation or combined R501X/2282del4 mutation were highly comparable with those of healthy controls (0–2.6% vs. 1.2–3.7%; Table 3).

Association between *FLG* loss-of-function mutations and characteristics of patients with atopic dermatitis

We also evaluated the association of *FLG* mutations with the duration of AD. In this subgroup analysis, in which only 145 patients with AD aged > 20 years at the time of analysis were

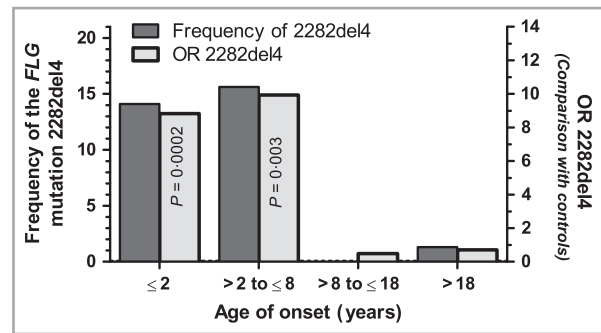


Fig 1. Frequencies and odds ratios (ORs) of the filaggrin gene (*FLG*) 2282del4 mutation analysed according to onset of atopic dermatitis development.

included, we identified an association between *FLG* mutation carriers and increased duration (≥ 20 years) of AD symptoms (Table 4). The association was statistically significant with *FLG* mutation carriers and AD duration ≥ 20 years compared with patients with earlier AD resolution (< 20 years) (2282del4, 22% vs. 1%, $P < 0.001$; OR 27.50, 95% CI 3.43–220.5; combined genotype R501X/2282del4, 24% vs. 2%, $P < 0.001$, OR 15.24, 95% CI 3.25–71.45) and with healthy controls (2282del4, $P < 0.001$; OR 15.54, 95% CI 4.13–58.44; combined R501X/2282del4, $P < 0.001$; OR 8.54, 95% CI 3.01–24.15). In contrast, no differences in *FLG* mutation frequencies were observed between patients with AD with a shorter duration of AD (< 20 years) and healthy controls.

We also divided patients with AD according to their history of hospitalization. In this subanalysis, we identified a strong association between both the *FLG* mutation 2282del4 ($P = 0.004$; OR 4.28, 95% CI 1.54–11.87) and the combined R501X/2282del4 mutation ($P = 0.007$; OR 3.27, 95% CI 1.40–7.64) and patients who required hospitalization compared with those who did not (Table 4). The association was even stronger when hospitalized patients were compared with control subjects (2282del4, $P < 0.001$; OR 9.07, 95% CI

Table 3 *FLG* mutations according to disease onset in patients with atopic dermatitis

Onset age (no. patients)	Comparison	FLG variant					
		2282del4			R501X or 2282del4		
		n (%)	OR (95% CI)	P-value	n (%)	OR (95% CI)	P-value
≤ 2 years (85)	Onset > 2 years	12 (14)	4.11 (1.48–11.39)	0.008	15 (18)	3.13 (1.34–7.32)	0.008
	Controls		8.82 (2.42–32.22)	< 0.001		5.64 (2.10–15.15)	< 0.001
> 2 years (156)	Onset > 5 years	6 (3.8)		NS	10 (6.4)		NS
	Controls	15 (15)	8.12 (2.28–28.88)	< 0.001	18 (18)	4.20 (1.68–10.50)	0.002
≤ 5 years (100)	Onset > 8 years	3 (2.1)		NS	7 (5.0)		NS
	Controls	17 (14.5)	9.47 (2.67–33.64)	< 0.001	22 (18.8)	5.78 (2.21–15.13)	< 0.001
> 5 years (141)	Onset > 18 years	1 (0.8)		NS	3 (2.4)		NS
	Controls	1 (1)	20.91 (2.73–159.9)	< 0.001	2 (3)	9.34 (2.71–32.15)	< 0.001
> 8 years (124)	Onset > 18 years	1 (1)		NS	2 (3)		NS
	Controls	3 (1.8)	9.12 (2.61–31.93)	< 0.001	6 (3.7)	6.10 (2.39–15.58)	< 0.001
Healthy controls (164)		3 (1.8)			6 (3.7)		

OR, odds ratio; CI, confidence interval; NS, statistically not significant.

Table 4 Association between FLG mutations and characteristics of patients with atopic dermatitis

Characteristic (no. patients)	Comparison	FLG variant					
		2282del			R501X or 2282del4		
		n (%)	OR (95% CI)	P-value	n (%)	OR (95% CI)	P-value
Duration ≥ 20 years (49)	< 20 years	11 (22)	27.50 (3.43–220.5)	< 0.001	12 (24)	15.24 (3.25–71.45)	< 0.001
	Controls		15.54 (4.13–58.44)	< 0.001		8.54 (3.01–24.15)	< 0.001
Duration < 20 years (96)	Controls	1 (1)		NS	2 (2)		NS
Hospitalization (83)	Not hospitalized	12 (14)	4.28 (1.54–11.87)	0.004	15 (18)	3.27 (1.40–7.64)	0.007
	Controls		9.07 (2.48–33.15)	< 0.001		5.81 (2.16–15.61)	< 0.001
No hospitalization (158)	Controls	6 (3.8)		NS	10 (6.3)		NS
Hospitalization and onset ≤ 5 years (60)	Others	12 (20)	7.29 (2.60–20.44)	< 0.001	15 (25)	5.70 (2.40–13.54)	< 0.001
	Controls		13.42 (3.64–49.52)	< 0.001		8.78 (3.22–23.94)	< 0.001
No hospitalization and onset > 5 years (181)	Controls	6 (3.3)		NS	10 (5.5)		NS
Onset ≤ 5 years and hospitalization and duration ≥ 20 years (24)	Others	8 (33)	14.63 (3.95–54.17)	< 0.001	9 (38)	13.92 (4.12–47.09)	< 0.001
	Controls		26.83 (6.47–111.4)	< 0.001		15.80 (4.95–50.46)	< 0.001
Onset > 5 years, no hospitalization and duration < 20 years (121)	Controls	4 (3.3)		NS	5 (4.1)		NS
Healthy controls (164)		3 (1.8)			6 (3.7)		

OR, odds ratio; CI, confidence interval; NS, not significant.

2.48–33.15; R501X/2282del4, $P < 0.001$; OR 5.81, 95% CI 2.16–15.61). Importantly, among the carriers of the FLG 2282del4 mutation, 66% of patients required at least one hospitalization due to AD. In addition, the median age of AD onset was < 2 years (1.75 years), and the median duration of the disease was 21.5 years (including four children who were younger than 8 years at the time of study inclusion).

Patients were also analysed according to the combination of AD disease onset, history of hospitalization, and disease duration. In this pooled analysis, we identified strong associations with the subgroups of onset age ≤ 5 years and hospitalization; or onset age ≤ 5 years, hospitalization and duration ≥ 20 years; and both 2282del4 or combined R501X/2282del4 compared with other patients ($P \leq 0.001$ for both) or control subjects ($P < 0.001$ for both; Table 4). No differences between controls and patients were noted for the subgroups onset age > 5 years and no hospitalization; and onset age > 5 years, no hospitalization and duration < 20 years.

In addition, the FLG mutation 2282del was more common among patients with AD with concomitant asthma than in patients without asthma (12% vs. 6.0%). However, this difference was not statistically significant ($P = 0.15$; OR 2.20, 95% CI 0.81–5.98). Nevertheless, a significant association between the FLG mutation 2282del4 and patients with AD and concomitant asthma was noted compared with control subjects ($P = 0.003$; OR 7.51, 95% CI 1.87–30.15).

Statistical power calculations performed under the dominant genetic model for carriers of the FLG loss-of-function mutations have revealed that in every analysis performed in

our study, the statistical power to detect a moderate association was at least 80%.

Discussion

This study revealed that FLG loss-of-function mutations are associated with the development of early-onset AD but not the development of late-onset AD, ACD or CICD. Furthermore, in addition to early onset, FLG mutations are also associated with an increased AD duration and the need for hospitalization.

We analysed the four most commonly described FLG mutations in the European white population. Similarly to other studies from central and south-eastern Europe,^{10,11,27} the most prevalent mutation in our study was 2282del4. This observation is in contrast to studies on north-western European populations, which have shown that the most prevalent mutation is R501X.^{2,5,6,28} Overall, we identified an association of AD with 2282del4 and with the combined R501X/2282del4 genotype, with ORs of 4.33 and 3.18, respectively. These ORs are comparable with the overall OR estimated in previous meta-analyses.^{29–31}

AD, which commonly presents during infancy and early childhood, can also begin in late childhood or adulthood in up to 20% of patients.^{12–14} Therefore, in contrast to previous FLG studies, which focused on the early onset of AD, our study evaluated the importance of these mutations for late AD development. Thus, one-third of our study population consisted of patients with infancy onset of AD (≤ 2 years), one-third was proportionally allocated to early-to-late-child-

hood onset of AD (2–18 years) and one-third of patients experienced adult-onset AD (> 18 years). This specific design allows for a nonbiased analysis of the association between FLG mutations and AD onset, which was not performed in any previous study to our knowledge.

The major finding was that FLG mutations were associated with only early-onset AD and not late-onset AD. Furthermore, we established a threshold of the age at which FLG mutations are still associated with AD development (≤ 8 years, with an OR up to 20.91 for the subgroup of 117 patients who developed AD during this age period). On the other hand, in the subgroup of 124 patients who developed AD when they were aged > 8 years, the frequencies of FLG mutations were similar to the low level observed in healthy controls. Importantly, the 8-year-old cut-off was chosen after analysis of the data, rather than being predefined. These observations may have important research and clinical implications.

Firstly, all FLG studies should be carefully designed according to the pattern of AD development, because we clearly demonstrated that the joint analysis of patients with varying disease onsets creates a significant bias. Secondly, further studies focusing exclusively on late-onset AD are obviously needed, given our results that demonstrated that the disease is not associated with epidermal barrier abnormalities due to loss-of-function FLG mutations in patients with onset of AD in late childhood or adulthood. The interaction of currently unknown susceptibility genes, acquired barrier defects and/or specific immunological dysregulation or inflammation features may be specifically associated with the development of late-onset AD. For example, a recent genome-wide association meta-analysis confirmed that polymorphisms in OVOL1 and ACTL9, which have been implicated in epidermal proliferation, as well as polymorphisms in IL4/KIF3A and IL6R, which are related to a cytokine cluster, may also be associated with AD.^{32,33} It has recently been demonstrated that IL6R functional variants specifically predispose individuals to persistent AD.³³

As previously reported,^{6,34–37} we also demonstrated the association of FLG mutations with AD duration and the severity of the clinical course of AD. This section has limitations. We were able to include only approximately half of the patients with AD for the persistence analyses. In addition, we did not include clinical severity score (Scoring Atopic Dermatitis) values for the severity analysis, because these values were not obtained for all patients. Therefore, the severity of the clinical course was estimated according to more frequent hospitalizations. In addition, we also combined AD development data with persistence and/or hospitalization data, and these analyses suggest that FLG mutations were associated with an 'early-onset persistent and/or severe' pattern. Nevertheless, a persistent and/or severe pattern was also observed in patients with late-onset AD. Thus, further studies are needed to confirm this 'pattern' observation.

FLG mutation carriers may also have an increased risk of other common complex skin and allergic diseases, including

ACD, CICD, asthma, hay fever and peanut allergy. In our study, we also focused on ACD and CICD. For ACD, previous studies demonstrated an association only with a subgroup of patients with nickel allergy,^{15,16} and not with unselected patients with ACD.^{15–19} In our study, we also did not observe any association of FLG mutations with unselected patients with ACD, which is consistent with previous studies. For CICD, a recent report demonstrated an increased susceptibility in individuals with concurrent FLG mutations and atopy²¹ or AD.²² However, when we assessed unselected patients with CICD, we did not observe any correlation with FLG mutations. Further studies are needed to determine whether FLG loss-of-function mutations are an independent risk factor for CICD.

In conclusion, our study reveals that FLG loss-of-function mutations are a predisposing factor exclusively for the development of early-onset AD. The observation of high FLG dependence for early AD development may result in an additional emphasis on these patients regarding the importance of adherence to specific therapeutic interventions for barrier improvement, with regular use of emollients or other topical therapies. In contrast, patients with AD with onset in late childhood or adulthood do not appear to be predisposed to skin barrier defects due to FLG mutations. The interaction of other susceptibility genes with the microenvironment and/or macroenvironment of the host, or even acquired barrier defects, is potentially important for the development of late-onset AD. Clearly, further studies should focus on the identifying risk factors for the development of AD in late childhood or adulthood.

References

- Scharschmidt TC, Man MQ, Hatano Y *et al.* Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. *J Allergy Clin Immunol* 2009; **124**:496–506.
- Irvine AD, McLean W, Leung DY. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med* 2011; **365**:1315–27.
- Chen H, Common JE, Haines RL *et al.* Unique and recurrent mutations in the filaggrin gene in Singaporean Chinese patients with ichthyosis vulgaris. *J Invest Dermatol* 2008; **128**:1669–75.
- Giardina E, Paolillo N, Sinibaldi C, Novelli G. R501X and 2282del4 filaggrin mutations do not confer susceptibility to psoriasis and atopic dermatitis in Italian patients. *Dermatology* 2008; **216**:83–4.
- Palmer CN, Irvine AD, Terron-Kwiatkowski A *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006; **38**:441–6.
- Barker JN, Palmer CN, Zhao Y *et al.* Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. *J Invest Dermatol* 2007; **127**:564–7.
- Ruether A, Stoll M, Schwarz T *et al.* Filaggrin loss-of-function variant contributes to atopic dermatitis risk in the population of Northern Germany. *Br J Dermatol* 2006; **155**:1093–4.
- Weidinger S, Illig T, Baurecht H *et al.* Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. *J Allergy Clin Immunol* 2006; **118**:214–19.

- 9 Ekelund E, Liedén A, Link J *et al.* Loss-of-function variants of the filaggrin gene are associated with atopic eczema and associated phenotypes in Swedish families. *Acta Derm Venereol* 2008; **88**:15–19.
- 10 Greisenegger E, Novak N, Maintz L *et al.* Analysis of four prevalent filaggrin mutations (R501X, 2282del4, R2447X and S3247X) in Austrian and German patients with atopic dermatitis. *J Eur Acad Dermatol Venereol* 2010; **24**:607–10.
- 11 Ponińska J, Samolińska B, Tomaszewska A *et al.* Filaggrin gene defects are independent risk factors for atopic asthma in a Polish population: a study in ECAP cohort. *PLoS ONE* 2011; **6**:e16933.
- 12 Schmitt J, Bauer A, Meurer M [Atopic eczema in adulthood]. *Hautarzt* 2008; **59**:841–50 (in German).
- 13 Hanifin JM, Reed ML. A population-based survey of eczema prevalence in the United States. *Dermatitis* 2007; **18**:82–91.
- 14 Schneider L, Tilles S, Lio P *et al.* Atopic dermatitis: a practice parameter update 2012. *J Allergy Clin Immunol* 2013; **131**:295–9.
- 15 Novak N, Baurecht H, Schäfer T *et al.* Loss-of-function mutations in the filaggrin gene and allergic contact sensitization to nickel. *J Invest Dermatol* 2008; **128**:1430–5.
- 16 Thyssen JP, Johansen J, Linneberg A *et al.* The association between null mutations in the filaggrin gene and contact sensitization to nickel and other chemicals in the general population. *Br J Dermatol* 2010; **162**:1278–85.
- 17 Carlsen BC, Thyssen JP, Menné T *et al.* Association between filaggrin null mutations and concomitant atopic dermatitis and contact allergy. *Clin Exp Dermatol* 2011; **36**:467–72.
- 18 Carlsen BC, Johansen JD, Menné T *et al.* Filaggrin null mutations and association with contact allergy and allergic contact dermatitis: results from a tertiary dermatology clinic. *Contact Dermatitis* 2010; **63**:89–95.
- 19 Lerbaek A, Bisgaard H, Agner T *et al.* Filaggrin null alleles are not associated with hand eczema or contact allergy. *Br J Dermatol* 2007; **157**:1199–204.
- 20 de Jongh CM, Khrenova L, Verberk MM *et al.* Loss-of-function polymorphisms in the filaggrin gene are associated with an increased susceptibility to chronic irritant contact dermatitis: a case-control study. *Br J Dermatol* 2008; **159**:621–7.
- 21 Landeck L, Visser M, Skudlik C *et al.* Clinical course of occupational irritant contact dermatitis of the hands in relation to filaggrin genotype status and atopy. *Br J Dermatol* 2012; **167**:1302–9.
- 22 Visser MJ, Landeck L, Campbell LE *et al.* Impact of atopic dermatitis and loss-of-function mutations in the filaggrin gene on the development of occupational irritant contact dermatitis. *Br J Dermatol* 2013; **168**:326–32.
- 23 Darsow U, Wollenberg A, Simon D *et al.* ETFAD/EADV eczema task force 2009 position paper on diagnosis and treatment of atopic dermatitis. *J Eur Acad Dermatol Venereol* 2010; **24**:317–28.
- 24 Sandilands A, Terron-Kwiatkowski A, Hull PR *et al.* Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 2007; **39**:650–4.
- 25 Sergeant A, Campbell LE, Hull PR *et al.* Heterozygous null alleles in filaggrin contribute to clinical dry skin in young adults and the elderly. *J Invest Dermatol* 2009; **129**:1042–5.
- 26 Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003; **19**:149–50.
- 27 Sabolic Pipinic I, Varnai VM, Turk R *et al.* Low frequency of filaggrin null mutations in Croatia and their relation with allergic diseases. *Int J Immunogenet* 2013; **40**:192–8.
- 28 Gruber R, Janecke AR, Grabher D *et al.* Lower prevalence of common filaggrin mutations in a community sample of atopic eczema: is disease severity important? *Wien Klin Wochenschr* 2010; **122**:551–7.
- 29 Baurecht H, Irvine AD, Novak N *et al.* Toward a major risk factor for atopic eczema: meta-analysis of filaggrin polymorphism data. *J Allergy Clin Immunol* 2007; **120**:1406–12.
- 30 van den Oord RA, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. *BMJ* 2009; **339**:b2433.
- 31 Rodríguez E, Baurecht H, Herberich E *et al.* Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. *J Allergy Clin Immunol* 2009; **123**:1361–70.
- 32 Paternoster L, Standl M, Chen CM *et al.* Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. *Nat Genet* 2011; **44**:187–92.
- 33 Esparza-Gordillo J, Schaarschmidt H, Liang L *et al.* A functional IL-6 receptor (IL6R) variant is a risk factor for persistent atopic dermatitis. *J Allergy Clin Immunol* 2013; **132**:371–7.
- 34 Carson CG, Rasmussen MA, Thyssen JP *et al.* Clinical presentation of atopic dermatitis by filaggrin gene mutation status during the first 7 years of life in a prospective cohort study. *PLoS ONE* 2012; **7**:e48678.
- 35 Margolis DJ, Apter AJ, Gupta J *et al.* The persistence of atopic dermatitis and filaggrin (FLG) mutations in a US longitudinal cohort. *J Allergy Clin Immunol* 2012; **130**:912–17.
- 36 Thyssen JP, Carlsen B, Menné T *et al.* Filaggrin null mutations increase the risk and persistence of hand eczema in subjects with atopic dermatitis: results from a general population study. *Br J Dermatol* 2010; **163**:115–20.
- 37 Brown SJ, Relton CL, Liao H *et al.* Filaggrin haploinsufficiency is highly penetrant and is associated with increased severity of eczema: further delineation of the skin phenotype in a prospective epidemiological study of 792 school children. *Br J Dermatol* 2009; **161**:884–9.