

Association between filaggrin null mutations and concomitant atopic dermatitis and contact allergy

B. C. Carlsen, J. P. Thyssen, T. Menné, M. Meldgaard,* A. Linneberg,† N. H. Nielsen,‡ P. B. Szecsi,* S. Stender* and J. D. Johansen

National Allergy Research Centre, Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Hellerup, Denmark; *Department of Clinical Biochemistry, Copenhagen University Hospital Gentofte, Hellerup, Denmark; †Research Centre for Prevention and Health, Glostrup Hospital, University of Copenhagen, Copenhagen, Denmark; and ‡Dermatology Clinic, Bagsværd, Denmark

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Summary

Background. The phenotypic traits of people with the filaggrin mutation (*FLG*) genotype and atopic dermatitis (AD) are still under elucidation, and the association with concomitant AD and contact allergy (CA) has not previously been examined.

Aim. To assess *FLG* status in a subset of patients with AD and a minimum of one positive patch-test reaction.

Methods. In total, 430 people from a hospital population and 3335 people from the general population were tested for *FLG* mutations by DNA hybridization to paramagnetic polystyrene beads and analysis on a multiplex analysis system. All of the individuals in the hospital population had a minimum of one CA. AD was diagnosed according to the UK Working Party Criteria, (questions-only version). Individuals from the hospital population who had both AD and CA were considered as cases, and comparison of mutation carrier frequency was estimated (χ^2 test) against individuals without AD but with CA from the hospital population, individuals from the general population, and individuals with AD from the general population.

Results. The mutation frequency in patients with AD and CA in the hospital population was significantly less than that of people with AD from the general population (OR = 0.54; 95% CI 0.30–0.98). No difference in mutation frequency was found between individuals with and without AD in the hospital population (OR = 1.40; 95% CI 0.70–2.79), or between individuals with AD and CA in the hospital population and in the overall general population (OR = 1.29; 95% CI 0.76–2.20).

Conclusions. The spectrum of observable traits characteristic for the *FLG* mutation genotype in patients with AD is at present not defined. Our results indicate that the subset of patients with both AD and CA represent a phenotype of AD that is not associated with *FLG* mutations.

Introduction

Filaggrin (FLG) is an essential structural protein of the skin barrier, which is produced in the upper layers of

the epidermis, where it crosslinks keratin filaments.¹ This process is a key part of the development of the skin barrier, which provides protection against environmental agents such as chemicals and microorganisms. FLG is also an important source of natural moisturizing factors in the skin, which regulate skin hydration.¹ To date, 22 null mutations in the *filaggrin* (*FLG*) gene have been detected in white populations.² These mutations lead to loss of production of FLG, resulting in increased transepidermal water loss, decreased hydration and increased thickness of the

Correspondence: Dr Berit Christina Carlsen, National Allergy Research Centre, Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, 2900 Hellerup, Denmark
E-mail: bccarlsen@dadlnet.dk

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stratum corneum, and increased penetration of allergens.^{3,4}

Atopic dermatitis (AD) is an itchy, inflammatory skin condition that varies widely in its onset, severity and course. At least 60% of cases have onset within the first year of life, and the majority within the first 5 years of life.⁵ About 40% of patients will have recurrent AD or persistence of disease into adulthood.⁵ Adult onset is also seen.⁶ AD predisposes to hand eczema, which is typically first seen in early adulthood.⁷

The underlying pathogenesis of AD is not fully understood. It was previously regarded as a disease driven primarily by the immune system.⁵ In 2006, it was reported that null mutations in the *FLG* gene were strongly associated with AD,⁸ shifting the understanding of the pathogenesis to a disease driven primarily by a structural skin barrier dysfunction followed by a secondary inflammatory response. Null mutations in the *FLG* gene are the single most important genetic factor for AD;⁹ however, not all patients with AD have *FLG* null mutations, and not all people with *FLG* null mutations will develop AD. The phenotypic traits of patients with AD and the *FLG* mutation genotype are still not clear. To date, the mutations have been associated with persistence of AD into adulthood,¹⁰ increased disease severity and early onset,¹¹ hyperlinearity of the palms, keratosis pilaris,¹² and perhaps a particular subtype of hand eczema.¹³

In this study, we aimed to assess the *FLG* null mutation status in a subset of patients of white ethnicity who had concomitant AD and contact allergy (CA) to at least one allergen.

Methods

The study was approved by the ethics committee of the capital region of Copenhagen, and the study was carried out in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment in the study.

Study population

In total, 1120 people, all with a minimum of one CA diagnosed by patch testing at the Department of DermatoAllergology (Copenhagen University Hospital Gentofte, Denmark) had previously participated in a questionnaire survey.¹⁴ Of these, 1057 were still alive and lived on Zealand and nearby islands. They were all asked to give a venous blood sample for *FLG* mutation detection; 432 people agreed to participate. Two people

were not of white ethnicity, and were excluded from further analyses, giving a final study population of 430 (96 men, 334 women; mean $\pm$ SD age 48 ± 13.6 years, range 14–87). Of these 430 participants, 157 had AD, and these constituted the case population.

A random sample of the general population in Copenhagen was used as controls, comprising 3471 people who had participated in a cross-sectional study for which a general health examination had been performed. This study population has previously been described in detail.¹⁵ Of these, 3335 (1491 men, 1844 women; mean $\pm$ SD age 48 ± 13.1 years, range 18–69) were successfully genotyped for R501X and 2282del4 *FLG* mutations.

Phenotype definition

The validated UK Working Party's Diagnostic Criteria (questions-only version) was used to identify individuals with AD in both cohorts.¹⁶ For the hospital population only, the question 'What year did the dermatitis first appear?' was used to determine the year of onset of (any) dermatitis; the age of onset was then calculated by subtracting the onset year from the year of birth.

Presence of hand eczema was specifically investigated in both groups. In the hospital population, the occurrence of hand eczema was determined by asking where the dermatitis was located at time of onset. The body surface was divided into 19 different skin areas in the questionnaire, one of the areas being the hands, including the wrists. Participants could choose any of these 19 different skin areas listed to identify the anatomical location(s) of the first dermatitis reaction they experienced. In the general population, the question 'Have you ever had hand eczema?' was used to determine the occurrence of the eczema.

Patch testing

To investigate the presence of CA, patch testing was carried out on the participants in the hospital population, using a patch-test series (TROLAB®; Hermal, Reinbek, Germany) in Finn chambers (Epitest Ltd Oy, Tuusula, Finland) attached to the skin with microporous tape (Scanpor®; Norgesplaster A/S, Alpharma, Vennesla, Norway) applied to the upper back. The occlusion time was 48 h. Readings were performed on day 2, day 3 or 4, and day 7, in accordance with the recommendations of the International Contact Dermatitis Research Group. A 1+, 2+, or 3+ reaction was interpreted as a positive reaction.

Genotyping

Regions covering the mutations R501X and 2282del4 of the filaggrin gene were amplified from genomic DNA by PCR, and the obtained DNA fragments were hybridized to both mutation-specific and wild-type-specific probes attached to fluorescent micro beads (Luminex, Austin, TX, USA). They were subsequently analysed on a multiplex analysis system (BioPlex 200; Biorad, Hercules, CA, USA). Validation of the method was performed by sequencing and parallel mutation determination at an independent laboratory (a manuscript describing this method in more detail is in preparation; publication of the details has been delayed by a patenting process).

Statistical analysis

Statistical analyses were performed using SPSS software (version 15.0; SPSS Inc. Chicago, IL, USA) except for assessment of Hardy–Weinberg equilibrium, which was performed using the web version of Genepop (<http://genepop.curtin.edu.au/>). The frequencies of the R501X and 2282del4 mutations, respectively, were combined into one mutation carrier frequency. The combined mutation carrier frequencies between subgroups were compared by the χ^2 test. The genotype variant for each mutation was in Hardy–Weinberg equilibrium in both cohorts ($P > 0.05$).

Results

Of the 430 patients in the hospital population, 37 (8.6%) were *FLG* mutation carriers. Of this population, 424 had been successfully classified according to AD status; 157 (37.0%) of these had AD, either currently or previously. Stratification by AD status showed that 16 patients (10.2%) with AD were *FLG* null mutation carriers, whereas 20 (7.5%) of patients without AD were also *FLG* null mutation carriers.

The clinical characteristics of the hospital population are given in Table 1, divided into the group with concomitant AD and CA, and the group without AD but with CA. Both groups were also stratified by age of onset of dermatitis: early onset < 15 years and late onset ≥ 15 years. More patients with AD had an early onset compared with patients without AD ($P < 0.001$); however, the majority of patients in both groups had a late onset, and hand eczema was the most common presenting feature.

The patient records of 24 of the 37 mutation carriers in the hospital population were reviewed to determine whether a physician diagnosis of AD had been made; the records of the remaining 13 mutation carriers could not be retrieved. The agreement between the diagnosis of AD made by a physician and the diagnosis of AD based on the UK Working Party Criteria is given in Table 2. There was disagreement between the diagnoses for two patients, and for eight patients, no records or indications of current or past AD were registered in the patient records.

In the control general population, 269 (8.1%) had a *FLG* mutation; 7 were homozygote and 260 were heterozygote for either R501X or 2282del4; 324 (9.7%) had present or previous AD. Stratification by AD status showed that 56 (17.3%) of people with AD were *FLG* mutation carriers, whereas 201 (7.0%) of people without AD were *FLG* mutation carriers. Clinical characteristics of the control population are given in Table 1.

The mutation frequency in patients with AD in the hospital population was compared with three different groups: (i) patients without AD but with CA in the hospital population; (ii) the overall mutation carrier frequency in the general population, and (iii) people with AD from the general population, (Table 3). The mutation carrier frequency in patients with AD and CA in the hospital population was no different from that in patients without AD in the hospital population, nor

Table 1 Clinical characteristics and mutation status for the general population and subgroups in both the hospital and general population.

	Hospital population		General population	
	AD	No AD	All	AD
F : M, <i>n</i> (%)	127 : 30 (80.9 : 19.1)	202 : 65 (75.7 : 24.3)	1844 : 1491 (55.3 : 44.7)	233 : 91 (71.9 : 28.1)
Age in years	46.7 $\pm$ 13.8*	48.7 $\pm$ 13.6*	48 (39–58)†	44 (34.25–54.75)†
Onset, <i>n</i> (%)				
Early (< 15 years)	61/154 (39.6)	51/226 (22.6)	–	–
Late (≥ 15 years)	93/154 (60.4)	175/226 (77.4)	–	–
Hand eczema, <i>n</i> (%)	98/153 (64.1)	149/232 (64.2)	716/3335 (21.5)	163/324 (50.3)
<i>FLG</i> mutation frequency, <i>n</i> (%)	16/157 (10.2)	20/267 (7.5)	269/3335 (8.1)	56/324 (17.3)

*Mean $\pm$ SD; †median (interquartile range).

Table 2 Agreement between atopic dermatitis diagnosed by physician in patient charts and by the UK Working Party Criteria.

Patient no.	Physician diagnosis	UK Working Party Criteria
1	–	–
2	–	–
3	NA	–
4	+	+
5	–	+
6	–	–
7	NA	+
8	+	+
9	+	+
10	NA	–
11	NA	–
12	+	+
13	+	+
14	–	–
15	+	+
16	+	+
17	NA	–
18	NA	–
19	–	–
20	–	–
21	–	+
22	–	–
23	NA	+
24	NA	+

+, Atopic dermatitis; –, not atopic dermatitis; NA, not assessed.

Table 3 Comparison of mutation carrier frequencies between the hospital atopic dermatitis group and patients without atopic dermatitis in the hospital population, the general population, and people with atopic dermatitis in the general population, respectively.

Population	AD status	<i>FLG</i> mutation carrier frequency, (%)	OR (95% CI)*
Hospital	AD + CA	10.2	1.40 (0.70–2.79)
Hospital	CA only, no AD	7.5	
Hospital	AD + CA	10.2	1.29 (0.76–2.20)
General	All	8.1	
Hospital	AD + CA	10.2	0.54 (0.30–0.98)†
General	AD only	17.3	

AD, atopic dermatitis; CA, contact allergy. * χ^2 Test; †significant.

from the overall mutation carrier frequency found in the general population; however, it was significantly lower than for people with AD from the general population.

Discussion

We found that patients with concomitant AD and CA to at least one allergen did not have a significantly higher

prevalence of *FLG* null mutations compared with patients with CA without AD, or compared with the general population. In fact, patients with both AD and CA had a lower frequency of mutations compared with people with AD from the general population. Thus, AD was not associated with *FLG* null mutations in the present hospital patch-test population. Previous studies have firmly established *FLG* null mutations as a risk factor for AD.¹¹ The mutation frequency in these studies was around 28% in hospital populations with mixed disease severities,¹⁷ and around 18% in community-based studies.¹² The differences in mutation frequency between studies are probably caused by different phenotype compositions and disease severities. Of note, the overall *FLG* mutation frequency in our study was 8.6% for the hospital population, 8.1% for the general population, and 10.2% for patients with AD and CA in the hospital population.

Studies have shown that certain phenotypes of AD are strongly associated with *FLG* null mutations. These subgroups include moderate to severe AD, early onset AD, and AD persisting into adulthood.^{10,11} Asthma in combination with eczema, but not asthma alone, is also associated with *FLG* mutations.¹¹ Some studies also suggest an increased association between *FLG* null mutations and extrinsic-type AD.¹⁸ Other studies have not been able to find differences in the strength of association between *FLG* null mutations and, respectively, intrinsic and extrinsic AD.¹⁹ Not all people who carry *FLG* null mutations will develop AD,²⁰ and evidently, not all patients with AD have *FLG* null mutations.

These data, along with the differences in mutation frequencies between studies, indicate that only specific subgroups of subjects with AD have an association with *FLG* null mutations. Our population of AD (characterized by CA to at least one allergen) may not be one of them. Our participants were all adults, with the majority having a late onset of dermatitis and the majority presenting as hand eczema at onset. Hand eczema is characteristic of the adult version of AD.⁶ The late onset and the domination of hand eczema may reflect that our population was comprised mainly of patients with adult onset AD, or a combination of patients with adult onset AD and patients with relapse in adulthood. Most studies performed to date on *FLG* null mutations and AD have been performed with children.

Around 16–45% of patients with clinical skin lesions consistent with AD do not have other atopic diseases such as rhinitis, asthma, food allergy or type I sensitizations.^{21,22} They also often do not have increased IgE.^{21,22} Such patients may be regarded as having

intrinsic AD, a subtype of AD that often has a late onset.²² A high IgE level is associated with a reduced ability to become sensitized to contact allergens; patients with low IgE are more easily sensitized under experimental conditions,²³ giving further support to the assumption that our population is dominated by people with an intrinsic type of AD with late onset. *FLG* null mutations have been associated in some studies with the extrinsic type of AD;¹⁸ however, consensus on this observation is not uniform. Furthermore, a low IgE level is associated with mild disease, whereas a high IgE level is associated with severe disease,²³ and the ability to develop CA is inversely related to severity of AD.²⁴ *FLG* null mutations are particularly associated with severe disease.¹¹ The patients with AD in our study population may be the subgroup of patients with AD with mild to moderate disease, which could explain the lack of association between *FLG* null mutations and AD. All of the aforementioned observations suggest that adult patients with AD and with CA represent a particular subgroup, with which *FLG* null mutations may not be associated.

Recently, a family-based study found a *FLG* mutation frequency of only 8.3%, despite the fact that the cases were selected based on a diagnosis of AD.²⁵ Not all people with AD are *FLG* mutation carriers. The phenotypic composition of the population in the Muller *et al.* study²⁵ may be uniquely different from similar studies, resulting in the surprisingly low mutation frequency.

We used the UK Working Party's Criteria, questions-only version, to assess the occurrence of AD. These criteria have been validated in adult and hospital populations.²⁶ Validation of these criteria was performed on consecutive adult outpatients including patients with AD, dermatitis and other dermatoses. AD was therefore assessed in relation to other dermatoses, which could easily be confused with AD,²⁶ and mimics the circumstances in this study. Some false-positive and false-negative cases are likely. However, most false-positive cases in the validation process were assessed as inactive cases of AD.²⁶ False-negative cases are few, as the sensitivity was as high as 92%. Further, in our study, we examined the patient records in the hospital population to identify the level of agreement between a diagnosis of AD made by a physician and that based on the UK Working Party criteria. The patient records on AD and the questionnaire assessment of AD matched overall, with discrepancies found for only two patients, a level compatible with sensitivity and specificity rate for the UK Working Party criteria.

Limitations

Our study has some limitations. We performed genotyping for the two most prevalent *FLG* null mutations in white populations, R501X and 2282del4; other less prevalent mutations have been identified. It is possible that these other null mutations were unevenly distributed between cases and controls; however, the two mutations studied account for approximately 85% of all *FLG* null mutations in white populations, making it unlikely that rarer mutations would change the results.

Conclusion

In conclusion, AD and *FLG* null mutations did not seem to be associated in the present study in a subgroup of adult patients with AD and a minimum of one positive patch test reaction. This indicates that this particular subset of patients with AD and CA represent a phenotype of AD that is not associated with *FLG* mutations.

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What's already known about this topic?

- Null mutations in the *FLG* gene are the single most important genetic factor for AD; however, not all patients with AD have *FLG* null mutations, and not all people with *FLG* null mutations will develop AD.
- The phenotypic traits of people with the *FLG* mutation genotype and AD are still unclear.
- To date, *FLG* mutations have been associated with persistence of AD into adulthood, increased disease severity and early onset, hyperlinearity of the palms, keratosis pilaris and perhaps a particular subtype of hand eczema.
- The association with concomitant AD and CA has not previously been examined.

What does this study add?

- The current study assessed the *FLG* null mutation status in a particular subset of patients with AD and a minimum of one CA.
- The aim was to evaluate whether this particular subset represents a phenotype of AD that is associated with *FLG* mutations.
- The study adds to the understanding of which phenotypic traits of AD are associated with the *FLG* mutation genotype.

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