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Filaggrin genotype and skin diseases independent of atopic dermatitis in childhood

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Abstract

Background: Filaggrin gene (*FLG*) mutations compromise skin barrier functions, and increase risk of atopic dermatitis. We aimed to study effects on other skin diseases using unique data from the Danish registers.

Methods: *FLG* genotyping of a population-based sample of 1547 children with extracted DNA and information on skin diseases from the Danish National Birth Cohort and Health Register, with 18 years follow-up during years 1996-2013. Odds ratios (OR) and hazard ratios (HR) were estimated using logistic regression and Cox regression, respectively, and adjusted for physician-diagnosed atopic dermatitis.

Results: *FLG* mutations were associated with increased risk of dry skin (OR 1.9, CI 1.1-3.1), and a decreased risk of fungal skin infections at age <18 months (OR 0.2, CI 0.1-0.8). There was no association with wart treatments (HR 1.0, CI 0.6-1.7). *FLG* mutations were associated with an increased risk of atopic dermatitis (OR 3.3, CI 2.1-5.3), dermatology consultations for allergy or rash (HR 2.2, CI 1.4-3.5), basic dermatology consultations at age <5 years (HR 2.2, CI 1.7-2.9), urticaria at age <18 months (OR 2.9, CI 1.0-7.9), and other rash at age <18 months (OR 2.1, CI 1.2-3.8).

Conclusions: *FLG* mutations may predispose to skin disease in young children including urticaria, and rash not recognized as atopic dermatitis although equally frequent. In clinical practice, *FLG* genotyping may help indicate the use of moisturizers to reduce skin problems.

Key words: Atopic dermatitis; filaggrin; fungus; genotype; dry skin; warts

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INTRODUCTION

Filaggrin proteins are essential for the normal formation and barrier function of the skin.¹ Importantly, filaggrin degradation products help to maintain the skin's hydration, mildly acidic pH, and normal extracellular lipid formations, and have also been suggested to provide immune modulation and photoprotection.² Filaggrin is also expressed in the oral mucosa.³ The major impact of filaggrin deficiency on skin diseases was shown in 2006^{4, 5} with the discovery of common loss-of-function mutations in the filaggrin gene (*FLG*) that result in reduced filaggrin expression and ichthyosis vulgaris (IV), a semidominantly inherited skin disease characterized by dry, scaly skin and palmar hyperlinearity.⁵ In addition, the mutations strongly increase the risk of atopic dermatitis (AD), a common childhood condition characterized by dry, itchy skin and dermatitis in specific anatomical areas.⁴

The high prevalence of *FLG* mutations (e.g. 8-10% of Europeans),² together with the importance of filaggrin for the skin, suggest that these mutations likely could influence the onset or course of skin diseases early in life and be associated with other diseases in addition to AD and IV.¹ However, few studies have investigated this. For example, only one study has examined the impact of *FLG* mutations on dry skin independent of AD i.e. the impact among persons *with* and *without* a history of AD.⁶ An impact of *FLG* mutations has been reported for certain skin infections in active AD,^{7, 8} but many have been reported to have a role in eczema pathogenesis.⁹⁻¹¹ We took advantage of a population-based sample of children with available DNA and information on skin diseases collected since birth and studied possible associations between *FLG* genotype and skin diseases, independent of the history of physician-diagnosed AD.

METHODS

Study population

The study population consisted of children from a previous case-control study¹²⁻¹⁵ nested in the Danish National Birth Cohort (DNBC) with available DNA and detailed information on skin diseases (www.dnbc.dk). In the earlier study, living singletons born preterm (cases, n=1000, <36 weeks) and term (controls, n=1000, 40 weeks) from uncomplicated pregnancies were selected for genome-wide association study.¹² In our study, we included the 1751 (92%) children with DNA remaining; *FLG* genotyping was successful for 1547 (88%) children who were then eligible for inclusion (see Genotyping section below). Information on skin disease outcomes, described below, was obtained from the DNBC and the Danish National Health Service Register (DNHS) by using the unique personal identification number assigned to all Danish residents.¹⁶

Ethics

The study was approved by the Scientific Ethics Committee of the Capital City Region (Copenhagen) (j.nr. (KF) 01-471/94, j.nr. H-A-2007-0017), the Danish Data Protection Agency (j. nr. 2008-54-0472) and the DNBC Steering Committee (j. nr. 2013-24).

Genotyping

Genotyping of 4 extensively studied *FLG* mutation markers (R501X, 2282del4, S3247X, and R2447X) was performed by LGC Genomics Ltd (Herts, UK) using KASPTM technology (see table S1). We defined mutation carriers as children with >1 mutation, and wildtype (i.e., ancestral type) carriers as children with a wildtype call for all 4 markers; *FLG* genotyping for the remaining children were unsuccessful - this was mainly because 2282del4 had a lower call rate of 90%, compared to 97-98% for the other markers (see S1). Specifically excluding

carriers of this mutation from the study, however, did not change results materially (see results section).

Skin diseases

Danish National Birth Cohort

Between 1996 and 2002, the DNBC study enrolled pregnant women (101,042 pregnancies to 91,769 women), who then participated in telephone interviews in the 16th and 30th weeks of pregnancy and 6 and 18 months postpartum (www.dnbc.dk) Cord blood samples were collected at birth. Seven years after birth, the women filled out a questionnaire about the health of the child. Our outcomes are described fully in S2. At age 7 years, we considered the following outcomes: 1) a history of physician-diagnosed AD (used in earlier publications¹⁷), 2) dry skin, and 3) a history of itchy rash. At age 6 and 18 months (early outcomes), we considered the following: 1) a history of fungal infection, and 2) a group with early rash based on questions on AD (validated in earlier publication¹⁸), urticaria, and other rash, respectively.

Danish National Health Service Register

The DNHS contains administrative registration of all primary health care services delivered to persons in Denmark since 1990. The data includes the personal identification number, the provider's medical specialty, the type of service, and the calendar week the service was performed.¹⁹ The register is virtually complete because registration is required for providers to be reimbursed by the state, and the state reimburses for most services (available to everyone). The classification system for the services types is primarily constructed to ease the reimbursement, but is also used for research purposes. We identified dermatological services and constructed three outcomes (described fully in S3): 1) consultations with a dermatologist

for allergy or rash, 2) basic consultations with a dermatologist (i.e. 1st, 2nd, or later consultation for a skin problem), and 3) wart treatment in the general practice.

Covariates

Preterm birth was defined as gestational age <36 weeks at birth. As we found it unlikely that environmental factors precede and alter the occurrence of *FLG* mutations, we did not consider confounding variables.

Statistical analysis

The data were evaluated using odds ratios (OR) for the retrospectively collected DNBC data, and using hazard ratios (HR) for the prospectively collected DNHS data. The latter allowing evaluation of time-dependent covariates.

Associations between *FLG* genotype and prevalence of skin diseases at a given age (e.g. dry skin at age 7 years) were evaluated by OR estimated using logistic regression in PROC GENMOD in SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina). We adjusted the ORs for history of physician-diagnosed AD at the given age of interest. When investigating outcome subtypes, each subtype was compared with the non-case group, thereby approximating multinomial logistic regression by pairwise logistic regression. Subtype-specific ORs were compared to evaluate the distribution of subtypes by *FLG* genotype using Chi-square statistics.

Associations between *FLG* genotype and time to next skin disease or outcome (e.g. time to next basic dermatologist consultation) was evaluated by hazard ratios (HR) estimated using Cox regression with age as the underlying time-scale in PROC PHREG procedure in SAS. As

the same child could have several events, we analyzed the data as recurrent event data and estimated the variance using a sandwich estimator (i.e. a marginal model). Children were followed from the most recent skin disease event (or birthday, if there were no previous events) until date of the next skin disease event or February 10, 2014 (the end of follow-up). We adjusted for history of physician-diagnosed AD, which was treated as a time-dependent variable and updated at age 6 months, 18 months, and 7 years. Interactions between *FLG* mutations and covariates (previous consultations and age, both time-dependent) were evaluated by including interaction terms in the model. To take changes in registration policy into account (see S3), follow-up for rash consultations began in 2001, while follow-up for wart consultations ended in 2006.

RESULTS

The study population consisted of 1547 children, of whom 935 (60%) had DNBC information at age 6 and 18 months, 969 (63%) had DNBC information at age 7 years, and 627 (41%) had information at all three ages. The compliance rates were similar to those for the entire DNBC (60%, 63%, and 40% of the cohort completed follow-up at the respective ages). Overall, 54% of the study population were males. *FLG* mutations were present in 145 (9.4%) children; S1 shows the distribution of the four mutations.

Table 1 shows the ORs for history of AD at age 7 years by *FLG* genotype (n=969), confirming that *FLG* mutation carriers have a more than 3-fold higher risk of AD. Sensitivity analysis supported this finding (S4). The OR for AD without a physician-diagnosis was not significantly different (P=0.84). The risk increase was largest for early-onset AD persisting at age 7 years, but the magnitude of the association was not significantly different from those for late-onset and previous AD (P=0.39). *FLG* mutations were also associated with increased

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risk of itchy rash and dry skin – two cardinal symptoms for AD; the risk for dry skin, however, was independent of AD (S5).

Table 2 shows the OR for history of fungal infections at age 18 months by *FLG* genotype. *FLG* mutations were associated with a reduced risk of fungal infections overall (OR 0.6, 95% CI 0.4-1.0). However, after adjustment for AD, the association was no longer statistically significant (aOR 0.7, 95% CI 0.4-1.1). Using maternal information on duration, type, frequency, treatment, and onset, we observed a consistent risk decrease, except in strata of duration and type. For the later two, risks were close to unity for short-lasting infections <3 days (OR 1.1, 95% CI 0.6-2.1) and oral thrush alone (OR 0.9, 95% CI 0.5-1.5). In contrast, the risk was significantly decreased for longer-lasting infections >3 days (OR 0.5, 95% CI 0.3-0.9) and *skin* infections (OR 0.2, 95% CI 0.1-0.8), even after adjustment for AD.

Table 3 shows the HRs for treatment of warts by general practitioners by *FLG* genotype. There was no overall association between *FLG* mutations and the risk of being treated for warts (173 cases with 388 treatments, HR 1.0, 95% CI 0.7-1.5), regardless of whether the child had previously been treated for warts and irrespective of current age. The results were not materially different when adjusted for AD.

Table 4 shows HRs for consultations with a dermatologist by *FLG* genotype. *FLG* mutations were associated with higher rates of consultations for allergy and rash (HR 2.7, 95% CI 1.7-4.2), whereas the rate of basic dermatologic consultations was only modestly increased (HR 1.3, 95% CI 1.1-1.6). However, whereas rates of consultations for allergy and rash were increased in all age groups, rates of basic dermatologic consultations were increased in the

first 5 years of life but not thereafter. For both outcomes, the main results were similar in children with and without previous consultations, or after adjustment for AD.

In an additional analyses, we studied whether *FLG* mutations increased the risk of early rash other than AD (n=935). The total group having early rash included children with a history of AD (150 cases), urticaria (30 cases), other rash (154 cases), or combinations of the three categories (17 cases). *FLG* mutations were strongly associated not only with AD (OR 3.0, 95% CI 1.8-5.2) but also with urticaria (OR 2.9, 95% CI 1.0-7.9), other rash (OR 2.1, 95% CI 1.2-3.8), and combinations of the three (OR 1.9, 95% CI 0.4-8.7).

In robustness analyses, we observed that the direction of results was the same for preterm and term children separately, and when excluding 67 children with the 2282del5 mutation (S6). Similarly, adjusting for preterm birth yielded almost similar estimates for all results (data not shown).

DISCUSSION

Our results showed that, even independent of AD, *FLG* mutations are associated with dry skin and frequent dermatological consultations in young children. In contrast to the hypothesis of increased susceptibility to skin infections, we observed no such association for warts or fungal infections.

As reported previously, *FLG* mutations were strong risk factors for AD.^{20, 21} The risk was higher for persistent AD with onset in young children (<2y), as suggested in some studies,^{22, 23} but we did not find the difference to be significant at age 7 years. With regards to dry skin,

we confirmed a previous report of an *independent* association with *FLG* mutations,⁶ which has not been studied elsewhere.²⁴

Quite consistently, the risk of fungal infections in young children decreased in *FLG* mutation carriers. The decrease was particular for long-lasting (>3 days) and cutaneous infections. We acknowledge the potential for some misclassification because we could not be sure that all fungal infections were physician-confirmed. However, misclassification with AD would tend to falsely *increase* the risk estimate for fungal infection (since AD children more often have *FLG* mutations),⁴ which was clearly not the case. In addition, mothers very likely recognize *common* fungal infections, such as oral thrush and diaper dermatitis (mainly caused by *Candida albicans*). The same may apply to long- rather than short-lasting fungal infection, or a dermatitis *responding* to antifungal treatment. We partly addressed any effect of such difference in maternal recognition by testing multiple categories such as duration, type, frequency, treatment status, and onset age. However, we cannot exclude a chance finding. A protective effect is compatible with *FLG* mutation-carriage resulting in higher skin levels of anti-fungal cytokines, i.e. IL-17 and IL-1,²⁵ and a dry skin,⁶ that lack one of the basic requirements for fungal growth, namely moisture.²⁶ In IV, fungal infections have been suggested to be uncommon.²⁷ Overall, the results clearly do not support that *FLG* mutations *increase* the risk of fungal infections.

We observed no association between *FLG* mutations and wart treatments, which include resection, cryotherapy, and vortex/verucid application. Clinically, warts are believed to occur more frequently and be more disseminated in AD patients.²⁸ However, previous studies have yielded inconsistent findings and may have had methodological shortcomings due to recall bias and concurrent evaluation for warts and AD.²⁹ Our results, which are based on

prospectively registered wart treatments in children, do not support the idea that a dysfunctional skin barrier in carriers of *FLG* mutations increases susceptibility to warts or necessitates multiple wart treatments.

FLG mutations were associated with a 2-fold increased risk of consultations for any skin problems at ages 0-4 years but not later. In contrast, if the consultation specifically concerned allergy or rash the risk increase presented at all ages (0-18 years). Adjustment for history of AD had little effect, suggesting the consulted skin problem was often not related to AD although still associated with *FLG* mutations. In line with this, the maternal reports of very frequent early skin problems were apparently also not related to AD (i.e. reported as urticaria and other “non-AD” rashes) but still associated with *FLG* mutations. We believe these findings suggest *FLG* mutations may have a broader impact on skin disease in young children than previously thought. For example, previous studies could not differentiate between early AD and other rash, and we observed an impact of *FLG* mutations on early dermatologist consultations independent of AD.³⁰ Furthermore, unobserved IV is too rare to account for the risk increase we observed (IV prevalence: <1% at age 6-7 years).²⁴ In perspective, we speculate that if young children are referred to a dermatologist and have *FLG* mutations, the use of moisturizers might be beneficial to prevent further skin problems.³¹

Our study had several great advantages. The study was nested in a large national population-based birth cohort, and sensitivity analyses suggested that the overrepresentation of preterm births was not a problem. Recall bias for early outcomes was strongly reduced by using maternal interview information obtained early in childhood, and information registered weekly by dermatologists and general practitioners. The weekly follow-up allowed us to evaluate an association with exact age and recurrence of consultations for skin problems.

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In conclusion, *FLG* mutations were not associated with increased risk of skin infections such as warts or fungal infection. *FLG* mutations may predispose to other skin disease in young children including urticaria, and rash not recognized as atopic dermatitis although equally frequent. If patients have *FLG* mutations, the use of moisturizers might be beneficial to prevent further development of the skin problem.

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Table 1. Odds ratio of a history of atopic dermatitis at age 7 years according to filaggrin genotype (wildtype/mutation) among 969 children from the Danish National Birth Cohort, born 1996-2002.

Outcome	Wildtype (reference)		Mutation		OR (95% CI)
	Cases (%)	Non-Cases ^a	Cases (%)	Non-Cases ^a	
Atopic dermatitis^b	173 (19.5)	711	38 (44.7)	47	3.3 (2.1-5.3)
Physician-diagnosed	161 (18.2)	711	35 (41.2)	47	3.3 (2.1-5.3)
Not physician-diagnosed	12 (1.4)	711	3 (3.5)	47	3.8 (1.0-13.9)
Previous	118 (13.4)	711	24 (28.2)	47	3.1 (1.8-5.3)

Current ^c	55 (6.3)	711	14 (16.5)	47	3.9 (2.0-7.4)
Early onset (<2 years)	34 (3.9)	711	11 (12.9)	47	4.9 (2.3-10.3)
Late onset (>2 years)	21 (2.4)	711	3 (3.5)	47	2.2 (0.6-7.5)

a: Number of non-cases were the same for all analyses with the outcome atopic dermatitis (i.e. 711+47 non-cases). For example, the 161+35 cases of physician-diagnosed atopic dermatitis and the 12+3 cases of not physician-diagnosed were both compared with the 711+47 non-cases who never had any atopic dermatitis whether physician-diagnosed or not. In this way, multinomial logistic regression were approximated by using pairwise logistic regression.

b: Among non-cases, 6.2% had *FLG* mutations.

c: Atopic dermatitis with itchy rash recurring during a 6 months period within the last 12 months at age 7 years.

Table 2. Odds ratio (OR) of a history of fungal infections at age 18 months according to filaggrin genotype among 935 children from the Danish National Birth Cohort, born 1996-2002.

Outcome	Wildtype (reference)		Mutation		OR (95% CI) ^e	aOR (95% CI) ^f
	Cases (%)	Non-cases ^a	Cases (%)	Non-cases ^a		
Fungal infections^{b, c}	321 (38.3)	517	26 (28.6)	65	0.6 (0.4-1.0)	0.7 (0.4-1.1)
<i>Maximum duration^c</i>						
<3 days	95 (11.3)	517	13 (14.3)	65	1.1 (0.6-2.1)	1.1 (0.6-2.1)
>3 days	225 (26.9)	517	13 (14.3)	65	0.5 (0.3-0.9)	0.5 (0.3-0.9)
<i>Type(s)^d</i>						
Oral (thrush)	171 (20.4)	517	19 (20.9)	65	0.9 (0.5-1.5)	0.9 (0.5-1.5)
Skin	82 (9.8)	517	2 (2.2)	65	0.2 (0.1-0.8)	0.2 (0.1-0.8)
<i>Frequency (>3 days)^e</i>						
1-3 times	140 (16.8)	517	9 (9.9)	65	0.5 (0.3-1.1)	0.6 (0.3-1.2)
Ever >3 times	81 (9.7)	517	4 (4.4)	65	0.4 (0.1-1.1)	0.4 (0.1-1.1)
1-6 times	149 (17.9)	517	9 (9.9)	65	0.5 (0.2-1.0)	0.5 (0.3-1.1)
Ever >6 times	72 (8.6)	517	4 (4.4)	65	0.4 (0.2-1.3)	0.5 (0.2-1.3)
<i>Ever treated</i>						
No	52 (6.2)	517	3 (3.3)	65	0.5 (0.1-1.5)	0.4 (0.1-1.5)
Yes	269 (32.1)	517	23 (25.3)	65	0.7 (0.4-1.1)	0.7 (0.4-1.2)
<i>Onset</i>						
Age <6 months	253 (30.2)	517	21 (23.1)	65	0.7 (0.4-1.1)	0.7 (0.4-1.1)

Age 6-18 months	68 (8.1)	517	5 (5.5)	65	0.6 (0.2-1.5)	0.6 (0.2-1.5)
Fungal infections according to AD						
No AD	282 (33.7)	447	23 (33.8)	65	0.8 (0.5-1.4)	-
AD	39 (35.8)	70	3 (13.0)	65	0.3 (0.1-1.0)	-

a: Number of non-cases were the same for all analyses with the outcome “Fungal infection(s)” (i.e. 517+65). For example, the 95+13 cases with duration <3 days and the 225+13 cases with duration >3days were both compared with the 517+65 non-cases who had no fungal infection(s). In this way, multinomial logistic regression were approximated by using pairwise logistic regression.

b: Among non-cases who had no fungal infection(s), 11.2% had *FLG* mutations.

c: A number of children excluded due to missing information on history of fungal infection (n=6), maximum duration (n=1), and frequency (n=4). Using 2 decimals, the OR for fungal infection overall was 0.64 (0.40-1.04).

(continued)

d: Because the 2 types (oral thrush, other i.e. skin) were registered at the 6 months follow-up only, only children with infections before age <6 months were included. The Danish term correctly used for oral thrush was “Trøske”. The group “Skin” included children who had episode(s) of fungal infection other than oral thrush. Among those, 63% had no history of oral thrush (53 cases, 2 with *FLG* mutations) and 37% had such history (31 cases, 0 with *FLG* mutations).

e: P-values for same OR in the 2 strata were in order of appearance, i.e. duration, type, frequency, frequency, treatment, onset, and fungal infection(s) according to AD: 0.03, 0.03, 0.67, 0.89, 0.53, 0.81, and 0.09.

f: aOR adjusted for a history of physician-diagnosed atopic dermatitis at age 6 months and 18 months (AD).

Table 3. Hazard ratio (HR) of wart treatment at ages 0-10 years at general practitioners according to filaggrin genotype among 1547 children from the Danish National Birth Cohort, born 1996-2002, Denmark.

Outcome	Wildtype (reference)		Mutation		HR (95% CI)	aHR (95% CI) ^a
	Treat- ments	Pys	Treat- ments	Pys		
Wart treatment^b	351	9138	37	932	1.0 (0.7-1.5)	1.1 (0.8-1.7)
<i>Previous wart treatments</i>						
0	157	8771	16	367	1.0 (0.6-1.7)	1.0 (0.6-1.7)
1+	194	896	21	36	1.1 (0.5-2.4)	1.3 (0.6-2.9)
<i>Age (years)</i>						

0-4	134	6653	12	676	0.9 (0.5-1.6)	0.9 (0.5-1.7)
5-9	217	2485	25	256	1.1 (0.7-1.8)	1.2 (0.8-1.9)
10+	0	0	0	440	-	-

CI, confidence intervals; Pyrs, person-years at risk; aHR, HR adjusted for physician-diagnosed atopic dermatitis as time-dependent variable.

a: The P-values of the test for same aHR in the strata of previous wart treatments and age were 0.53 and 0.51, respectively.

b: 173 children (11.2%) had wart treatments at ages 0-10 years.

Table 4. Hazard ratio of consultations with a dermatologist at ages 0-18 years according to filaggrin genotype among 1547 children from the Danish National Birth Cohort, born 1996-2002.

Outcome	Wildtype (reference)		Mutation		HR (95% CI)	aHR (95% CI) ^d
	Consul- tations	Pyrs	Consul- tations	Pyrs		
Dermatologist consultations for allergy or rash^a						
(Any)	129	18404	36	1890	2.7 (1.7-4.2)	2.2 (1.4-3.5)
Allergy ^a	82	18130	26	1841	3.2 (1.8-5.6)	2.6 (1.5-4.6)
Rash ^a	47	15772	10	1596	2.2 (1.1-4.7)	1.8 (0.9-3.9)
Previous consultations						
0	57	18094	12	1812	2.2 (1.2-4.0)	1.8 (1.0-3.4)
1+	72	310	24	78	1.6 (0.7-3.6)	1.8 (0.8-3.9)
Age (years)						
0-4	39	6,572	14	630	3.2 (1.5-6.9)	2.7 (1.2-5.9)
5-9	46	7373	9	812	2.3 (1.0-5.3)	1.8 (0.8-4.2)
10+	44	4459	13	448	2.5 (1.2-5.4)	2.0 (1.0-4.3)
Basic dermatologist consultations^b						
(Any)	1424	18404	191	1890	1.3 (1.1-1.6)	1.3 (1.0-1.6)
Previous consultations						
0	438	15278	54	1409	1.3 (1.0-1.8)	1.3 (1.0-1.7)
1+	986	3126	137	481	0.9 (0.6-1.1)	0.9 (0.7-1.2)
Age (years)						
0-4 ^c	392	2,467	93	301	2.3 (1.8-2.8)	2.2 (1.7-2.9)
5-9	592	10736	60	1056	1.0 (0.7-1.3)	0.9 (0.7-1.3)
10+	440	5241	38	533	0.9 (0.6-1.2)	0.8 (0.6-1.2)

CI, confidence intervals; Pyrs, person-years at risk; aHR adjusted for physician-diagnosed atopic dermatitis as time-dependent variable.

a: 69 children (4.5%) had consultations at ages 0-18 years, of whom 5 had services for both allergy and rash. HRs for allergy and rash were estimated by considering only the specific consultation type as the outcome.

b: 492 children (31.8%) had consultations at ages 0-18 years.

c: The aHR for age 0, 1, 2, 3, and 4 years was 3.1, 3.7, 1.3, 1.5 and 1.5.

d: Tests for same HR in strata: For the outcome allergy or rash, P-value for same HR in the strata of previous consultations were 0.35. The corresponding P-value for age was 0.77. For the outcome basic consultations, the corresponding P-values was <0.001 for age, and 0.02 for previous consultations however 0.31 after adjustment for the age-specific effect.

S1. Genotyping and Hardy-Weinberg equilibrium for 4 markers of filaggrin gene mutations in 1751 children with DNA available from the Danish National Birth Cohort.

	OBSERVED GENOTYPES						EXPECTED GENOTYPES			HWE
Marker	wt/wt	wt/mt	mt/mt	N	Percent missing	Allele frequency mt	wt/wt	wt/mt	mt/mt	exact p-value
R501X	1636	61	0	1697	3.0%	0.018	1636.5	59.9	0.5	1
2282del4	1512	54	13	1579	9.7%	0.025	1500.0	78.0	1.0	1.30*10 ⁻¹²
S3247X	1706	8	0	1714	2.0%	0.002	1706.0	8.0	0.0	1
rs138726443	1716	11	0	1727	1.3%	0.003	1716.0	11.0	0.0	1
Study										
Inclusion	1402	134*	13*	1547						
Exclusion	204†									

Comment

Percentage of missing refers to (1751-N)/1751 %. Hardy-Weinberg equilibrium (HWE) was tested using an exact test to take into account type I error if the Chi-square test is used.¹ There was no significant deviations from HWE, except for 2282del4 (*P-value* = 1.30*10⁻¹²) - which was the only marker genotyped as homozygous (mt/mt).

*The 134 heterozygous and 13 homozygote children together sum up to 145, and not 147, children being mutation carriers because 2 children each carried 2 mutations (2282del4+R501X, and 2282del4+S3247X); the remaining carried only one mutation.

† Excluded from the study because 1-3 of the markers had a missing call, i.e. it was uncertain whether it was a wildtype or a mutation. This uncertainty was thereby removed from the study's reference group. However, the results for all main outcomes presented in S6 were very similar if including the 204 children in the study's reference group (data not shown).

Reference

(1) Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet 2005 May;76(5):887-93

S2. List of questions used to define the study's dermatological outcomes obtained from the Danish National Birth Cohort (DNBC).

All questions can be found at www.dnbc.dk translated to English. Mothers were telephone-interviewed during pregnancy (week 12 and 30), and when the child was aged 6 months (6m) and 18 months (18m), and filled in questionnaires at age 7 years (7y).

Outcomes	Age at follow-up: question number
<i>A history of atopic dermatitis (AD) at age 7 years</i>	7y: z041
Physician-diagnosed (phys.)	7y: z034
Current	7y: z034, z035, z036
Early/late onset	7y: z038
<i>A history of AD according to the UK working party criteria (slightly modified to fit the questions*)</i>	
Criteria:	
An itchy skin condition	7y: z035
plus minimum 2 of the following four criteria:	
1. Onset below age 2 years	6m: s104; 18m: s051
	7y: z034, z035, z036, z038
2. History of flexural involvement	7y: z037
3. History of a generally dry skin in the last 12 months	7y: z042
4. Personal history of asthma or hay fever	7y: z051 and z053
<i>A history of phys. AD at age 6m, 18m, and 7 years</i>	6m: s104; 18m: s051; 7y: z034
<i>Dry skin</i>	7y: z042
<i>Itchy rash</i>	7y: z035
<i>A history of fungal infection</i>	6m: c118-c129; 18m: d060-d063
<i>Early rash</i>	6m: c103, c108, c113; 18m: d050, d054, d056

Questions related to atopic dermatitis and rash:

c103 = d050: Did your child have atopic dermatitis**?

c108 = d054: Did your child have urticaria?

c113 = d056: Did your child have other kinds of eczema or skin rash/eruptions?

s104 = s051: Did a doctor say that your child had atopic dermatitis**?

z034: Has a doctor ever said that your child had atopic dermatitis**?

z035: Has your child ever had an itchy rash which was coming and going for at least 6 months?

z036: Has your child had this itchy rash (i.e. z036) at any time in the last 12 months?

z037: Has this itchy rash (i.e. z036) at any time affected the following places: the folds in the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?

z038: At what age did this rash (i.e. yes to z036) first occur? (<2 years, 2-4 years, 5 or more years)

z041: Has your child ever had eczema, also known as allergic rash?

z042: Does your child have dry skin?

z051: Has a doctor ever said that your daughter had asthma?

z053: Has a doctor ever said that your daughter suffers from hay fever?

* 2 of the 5 original criteria was merged into our criteria no. 2, because they are both covered by i.e. question z037, i.e. includes current (7y) and previous flexural involvement of the eczema (<7y) - although only for those with current rash at age 7 years (yes to z036). See reference in S4.

** Danish terms used: "børneeksem/astmaeksem" at age 6m+18m and "eksem, eller allergisk udslæt" at age 7 year.

S3. List of health services defining the study's dermatological outcomes obtained from the Danish National Health Service register (DNHS)

The number of services (z) provided for all children in the study population (n=1547) is shown for each service requested from the register. The resulting outcomes presented in the paper do not include services that were not provided to the study cohort (i.e. z=0). We chose services from the register's documentation by means of an electronic and systematic search for dermatological services. Note that the DNHS creates service numbers in excess so that reimbursement is possible even if the service is rare within the specialty (e.g. warts are rarely treated during a guard physician assistance, specialty no. 83).

The DNHSs historical overview covering 1990-2008 ([Ydelsesoversigt historisk](http://www.ssi.dk/Sundhedsdataogit/Registre%20og%20kliniske%20databaser/De%20nationale%20sundhedsregistre/Sundhedsokonomi%20finansiering/Sygesikringsregister.aspx)) can be found on: <http://www.ssi.dk/Sundhedsdataogit/Registre%20og%20kliniske%20databaser/De%20nationale%20sundhedsregistre/Sundhedsokonomi%20finansiering/Sygesikringsregister.aspx>

Outcome: Dermatologist consultations for allergy or rash

(Services possibly related to childhood atopic dermatitis under the Dermato-venereology specialty*)

Service number	Service text	Registration Period	Services (z) in the study
0240	Rash. 1 st consultation	2001-2008	31
0250	Rash. 2 nd consultation	2001-2008	10
0260	Rash. Later consultation	2001-2008	14
0270	Rash. Telephone consul.	2001-2008	2
0210	Allergy. 1 st consultation	1990-2008	39
0220	Allergy. 2 nd consultation	1990-2008	18
0230	Allergy. Later consultation	1990-2008	13
0310	Allergy. Phone consultations	1990-2008	0
2201	Allergy. Cutaneous test	1990-2008	38
2202	Allergy. Provocation experiment	1990-2008	0

Outcome: Basic dermatologist consultations

(Basic services under the Dermato-venereology specialty*)

Service number	Service text	Registration period	Services (z) in the study
0110	1 st consultation	1990-2008	713
0120	2 nd consultation	1990-2008	365
0130	Later consultation	1990-2008	695

(S3 continued)

Outcome: *Wart treatment*

(Services related to warts)

Speciality*	Service number	Service text	Registration period	Services (z) in the study
80	2103	Wart treatment	1990-2008	388
83	2103	Removal of warts	1990, 1992-93, 1995-2000, 2002-2006	0
04	5016	Laser treatment warts §3	2002-8	0
04	5020	Photodynamic therapy-treatment, wart patient	2003-5	0
04	5022	Photodynamic therapy-warts	2003-8	0

*Specialty numbers and names were as follows: 04, Dermato-venereology specialty; 80, General practices; 83, Guard physicians.

S4. Sensitivity analysis of the outcome atopic dermatitis

We performed 2 sensitivity analyses among the same children with completed 7-year follow-up questionnaires (n=969). In one analysis the outcome was a history of atopic dermatitis before age 6 months, 18 months or 7 years; here 30 additional cases were identified and added to the 211 already identified cases (OR 3.0, CI 1.9-4.8). In the other analysis the outcome was cases who fulfilled the UK working party criteria^{1, 2} (see S2) or had a history of atopic dermatitis before age 7 years; here 14 additional cases were identified and likewise added (OR 3.2, CI 2.0-5.0).

References

- (1) Williams HC, Burney PG, Pembroke AC, Hay RJ. Validation of the U.K. diagnostic criteria for atopic dermatitis in a population setting. U.K. Diagnostic Criteria for Atopic Dermatitis Working Party. *Br J Dermatol* 1996 Jul;135(1):12-7.
- (2) Breninkmeijer EE, Schram ME, Leeflang MM, Bos JD, Spuls PI. Diagnostic criteria for atopic dermatitis: a systematic review. *Br J Dermatol* 2008 Apr;158(4):754-65.

S5. Odds ratio (OR) of having dry skin or a history of itchy rash at age 7 years according to filaggrin genotype among 969 children from the Danish National Birth Cohort, born 1996-2002.

Outcome	Wildtype (reference)		Mutation		OR (95% CI)	aOR (95% CI) [§]
	Cases (%)	Non-cases*	Cases (%)	Non-cases*		
Dry skin or itchy rash[†]	293 (33.1)	591	49 (57.7)	36	2.8 (1.8-4.3)	1.9 (1.1-3.3)
Dry skin[‡]	257 (29.1)	627	44 (51.8)	41	2.6 (1.7-4.1)	1.9 (1.1-3.1)
Itchy rash^{††}	128 (14.5)	756	23 (27.1)	62	2.2 (1.3-3.7)	1.1 (0.6-2.1)
Dry skin according to AD^{‡‡}						
No AD	147 (20.3)	576	19 (38.0)	31	2.4 (1.3-4.4)	-
AD	110 (68.3)	51	25 (71.4)	10	1.2 (0.5-2.6)	-
Itchy rash according to AD^{‡‡}						
No AD	40 (5.5)	683	2 (4.0)	48	0.7 (0.2-3.0)	-
AD	88 (54.7)	73	21 (60.0)	14	1.2 (0.6-2.6)	-

AD, Atopic dermatitis

* Number of non-cases were children without the outcome under analysis. For example, the 293+44 cases of “dry skin or itchy rash” were compared with the 591+36 non-cases who had no record of dry skin or itchy rash.

[†] Among non-cases, 5.7% had *FLG* mutations.

[‡] Among non-cases, 6.1% had *FLG* mutations.

^{††} Among non-cases, 7.6% had *FLG* mutations.

^{‡‡} P-values for same OR in the 2 strata (i.e. test for interaction) were 0.02 for the dry skin and 0.57 for itchy rash.

[§] aOR, OR adjusted for a history of physician-diagnosed AD at age 7 years.

Text summary of results in the table:

S5 above shows the ORs for *dry skin* and *history of itchy rash* at age 7 years by *FLG* genotype. Overall, *FLG* mutations were associated with a 2- to 3-fold increased risk of both outcomes. However, after adjustment for AD, the increase in risk persisted for dry skin (OR 1.9 95% CI 1.1-3.1) but not for itchy rash (OR 1.1, 95% CI 0.6-2.1). Among children without and with a history of AD, the ORs for dry skin were 2.4 and 1.2 ($P_{\text{interaction}}=0.02$), respectively, and for itchy rash they were 0.7 and 1.2 ($P_{\text{interaction}}=0.57$), respectively.

S6. Robustness analyses.

Odds ratio or Hazard ratio of the association between filaggrin genotype and skin disease outcomes, stratified by term of birth, or excluding 67 children with the 2282del4 mutation.

Outcome	Term of birth (N=1547)		Excluding 2282del4 mutation carriers (N=1547-67=1480)
	Preterm OR, HR* (95% CI)	Term OR, HR* (95% CI)	OR, HR* (95% CI)
Atopic dermatitis (AD) <7y	3.0 (1.3-7.0)	3.5 (2.0-6.2)	3.8 (2.2-6.7)
Physician-diagnosed AD <7y	3.1 (1.3-7.4)	3.2 (1.8-5.7)	3.9 (2.2-6.8)
Dry skin	1.4 (0.6-3.2)	3.7 (2.1-6.5)	2.6 (1.5-4.6)
Itchy rash	1.9 (0.7-5.0)	2.3 (1.2-4.3)	2.4 (1.3-4.5)
Fungal infection	0.9 (0.4-2.1)	0.6 (0.3-1.0)	0.5 (0.3-1.0)
Wart treatment by G.P.*	1.2 (0.6-2.2)	1.0 (0.6-1.6)	1.1 (0.7-1.5)
Dermatologist, allergy/rash*	3.0 (1.5-5.9)	2.6 (1.4-4.8)	1.8 (0.9-3.6) [†]
Dermatologist, basic, 0-4 y*	2.3 (1.5-3.6)	2.3 (1.7-3.1)	2.5 (1.8-3.5)
Early rash (age<18 m)	4.8 (2.0-11.2)	2.0 (1.2-3.4)	2.7 (1.5-4.8)
AD	4.4 (1.6-12.3)	2.6 (1.4-5.1)	2.9 (1.4-5.9)
Urticaria	4.0 (0.8-21.1)	3.1 (0.8-11.5)	2.2 (0.5-9.9)
Other [‡]	5.1 (2.0-13.0)	1.5 (0.8-3.0)	2.7 (1.3-5.5)

* Outcomes based on DNHS data, i.e. data were evaluated using HR.

[†] Age-specific HR were 2.7 (0.9-7.7) for 0-4 years, 3.1 (1.2-8.0) for 5-10 years, and for 10+ years numbers were too small to calculate HR.

[‡] Children without a history of AD or urticaria.