

Report

Filaggrin gene loss-of-function mutations explain discordance of atopic dermatitis within dizygotic twin pairs

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Introduction

Loss-of-function mutations in the filaggrin gene (*FLG*) increase the risk for atopic dermatitis (AD) that is characterized by an early onset and persistent course. Moreover, *FLG* mutations in the context of AD increase the risk for developing allergic asthma, probably as a result of primary cutaneous sensitization and a secondary inflammatory response in the airway following airborne allergen exposure.¹

The rate of concordant AD in twins is consistently higher in monozygotic (MZ) than in dizygotic (DZ) twins,² indicating that genetic factors play a major role in the development of AD. Furthermore, twin studies have shown that genetic factors explain a significant proportion of the co-occurrence of AD and asthma.³ No twin study has yet examined the associations between AD, atopic diseases, and loss-of-function mutations in

Abstract

Objectives This study was designed to examine the association between loss-of-function mutations in the filaggrin gene (*FLG*) and atopic dermatitis (AD) and asthma in adult twins.

Methods A previously well-characterized cohort of 575 adult twins were genotyped for the loss-of-function mutations in *FLG* (R501X, 2282del4 and R2447X) most common among northern Europeans. Subjects were examined for symptoms of atopic diseases as well as for lung function, airway responsiveness, and atopy.

Results In the whole population of twins, the risk for AD was significantly increased in individuals with *FLG* mutations in comparison with wild-type carriers (34.3% vs. 21.8%) after adjustment for possible confounders (odds ratio [OR] 1.92, 95% confidence interval [CI] 1.07–3.41; $P = 0.028$). A significant association was also observed for persistent AD (OR 2.10, 95% CI 1.02–4.36; $P = 0.046$). There were no significant differences in risk for asthma by *FLG* mutation status in individuals with and without AD, respectively (P -value for interaction, 0.595). In 11 dizygotic twin pairs discordant for *FLG* mutation status, risk for AD was higher in the twin carrying the *FLG* mutation (five of 11 [45.5%] twins had developed AD) than in the non-carrier co-twin (two of 11 [18.2%] twins had developed AD) (OR 2.50, 95% CI 0.45–13.85; $P = 0.293$). *FLG* status did not explain a significant proportion of the variation in AD ($P = 0.328$) or asthma ($P = 0.321$).

Conclusions Filaggrin gene mutations are risk factors for the presence and persistence of AD and explain the discordance of AD within dizygotic twin pairs.

FLG. In the present investigation, a well-characterized cohort of adult twins⁴ were genotyped for the loss-of-function mutations in *FLG* most common among northern Europeans (R501X, 2282del4, and R2447X).⁵ The associations between these variants, AD and asthma and the proportion of variation, respectively, in AD and asthma susceptibility attributable to *FLG* mutations were examined.

Materials and methods

The cohort consisted of 575 twin individuals ascertained through the nationwide Danish Twin Registry⁶ and selected through a proband with self-reported asthma (at least one twin in each twin pair had asthma). Subjects comprised 89 MZ twin pairs, 167 DZ twin pairs, and 63 single twins. All subjects underwent a clinical examination and an interview about their

history of AD (in line with the UK Working Party's criteria⁷), asthma, and hay fever. The local scientific ethics committee approved the study. Informed consent was obtained from all participants in writing and verbally. Statistical analyses were conducted using SPSS for Windows Version 16.0 (SPSS, Inc., Chicago, IL, USA) and SOLAR Version 2.0 (Solar Software, Inc., Westerville, OH, USA).

Results

The mean age of the subjects was 35.9 years (range: 21–51 years), and 41.7% were men (Table 1). The lifetime prevalence of AD was 23.8%, whereas the prevalence of persistent AD (symptoms within the past 12 months) was 10.4%. A total of 70 individuals (12.2%) were *FLG* mutation carriers (three were homozygotes and none were compound heterozygotes).

In the whole population of twins, the risk for AD was significantly increased in individuals with *FLG* mutations in comparison with wild-type carriers (34.3% vs. 21.8%; odds ratio [OR] 1.87, 95% confidence interval [CI] 1.09–3.20; $P = 0.022$), even after adjustments for sex, age, twin zygosity, proband's asthma status, and co-twin's AD status (OR 1.92, 95% CI 1.07–3.41;

$P = 0.028$). A significant association was also observed for persistent AD (OR 2.10, 95% CI 1.02–4.36; $P = 0.046$) after adjustment for possible confounders.

The risk for AD was increased in individuals with asthma compared with individuals without asthma (27.6% vs. 18.5%; OR 1.68, 95% CI 1.12–2.52; $P = 0.012$). This was even more prominent for persistent AD (13.6% vs. 5.9%; OR 2.53, 95% CI 1.36–4.72; $P = 0.003$). However, there were no significant differences in risk for asthma according to *FLG* mutation carrier status in individuals with and without AD, respectively (P -value for interaction, 0.595).

The cumulative incidence of AD according to age of onset in *FLG* mutation carriers and non-carriers is depicted in Figure 1. The mean age of AD onset was lower in *FLG* mutation carriers than in non-carriers (5.5 years vs. 8.3 years; $P = 0.148$).

Among the 89 MZ pairs, a total of 13 pairs were concordant and 20 were discordant for AD (pairwise concordance rate: 0.39); among the 167 DZ pairs, 11 pairs were concordant and 57 were discordant for AD (pairwise concordance rate: 0.16) (P -value for the difference between MZ and DZ twins, 0.014). All twin pairs concordant for AD (both MZ and DZ pairs) were also concordant for the three examined *FLG* variants, whereas the group of DZ

Table 1 Descriptive characteristics of 575 adult twins

Characteristic	All ($n = 575$)	MZ twins ($n = 191$)	DZ twins ($n = 384$)	P -value
Age, years, mean $\pm$ SD	35.9 $\pm$ 8.2	34.9 $\pm$ 7.4	36.4 $\pm$ 8.5	0.022
Sex, n (%)				0.556
Male	240 (41.7%)	83 (43.5%)	157 (40.9%)	
Female	335 (58.3%)	108 (56.5%)	227 (59.1%)	
BMI, kg/m ² , mean $\pm$ SD	25.3 $\pm$ 4.6	25.0 $\pm$ 4.4	25.4 $\pm$ 4.7	0.318
Smoking ever, n (%)	262 (45.6%)	79 (41.4%)	183 (47.7%)	0.153
<i>Clinical traits</i>				
AD ever (all subjects), n (%)	137 (23.8%)	49 (25.7%)	88 (22.9%)	0.468
Subjects with <i>FLG</i> mutation, n (%)	24 (34.3%)	8 (38.1%)	16 (32.7%)	
Subjects without <i>FLG</i> mutation, n (%)	109 (21.8%)	41 (24.3%)	68 (20.6%)	
AD in past 12 months, n (%)	60 (10.4%)	30 (15.7%)	30 (7.8%)	0.004
Asthma (all subjects), n (%)	337 (58.6%)	126 (66.0%)	211 (54.9%)	0.012
Subjects with <i>FLG</i> mutation, n (%)	38 (54.3%)	15 (71.4%)	23 (46.9%)	
Subjects without <i>FLG</i> mutation, n (%)	296 (59.3%)	111 (65.7%)	185 (56.1%)	
Hay fever, n (%)	309 (53.7%)	120 (62.8%)	189 (49.2%)	0.002
<i>Paraclinical traits</i>				
Filaggrin mutation, n (%)	70 (12.2%)	21 (11.0%)	49 (12.8%)	0.507
R501X	28 (4.9%)	10 (5.2%)	18 (4.7%)	0.800
2282del4	34 (5.9%)	8 (4.2%)	26 (6.8%)	0.204
R2447X	9 (1.6%)	3 (1.6%)	6 (1.6%)	0.991
Positive skin prick test, n (%)	318 (55.3%)	113 (59.2%)	205 (53.4%)	0.189
Airway hyper-responsiveness, n (%)	149 (25.9%)	52 (27.2%)	97 (25.3%)	0.417
Airway obstruction, mean $\pm$ SD	0.84 $\pm$ 0.09	0.84 $\pm$ 0.10	0.84 $\pm$ 0.08	0.748
Serum total IgE, kU/L, median (range)	54 (1–7003)	54 (1–2342)	53 (1–7003)	0.271
Exhaled nitric oxide, ppb, median (range)	17 (3–260)	18 (4–181)	17 (3–260)	0.330

Airway obstruction is spirometrically measured forced expiratory volume in 1 s (FEV1) divided by functional vital capacity (FVC).

P -values refer to differences between MZ and DZ twins.

AD, atopic dermatitis; BMI, body mass index; DZ, dizygotic; *FLG*, filaggrin gene; IgE, immunoglobulin E; MZ, monozygotic; SD, standard deviation.

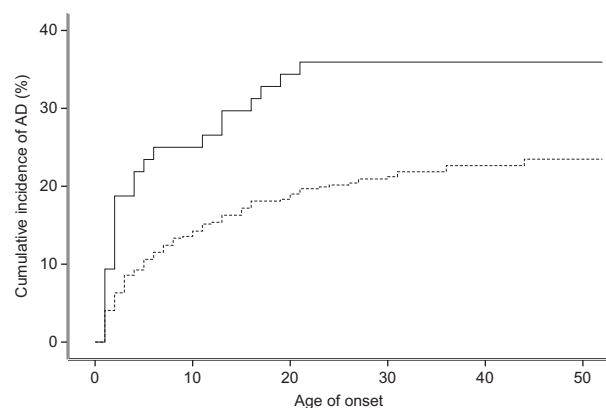


Figure 1 Risk for atopic dermatitis (AD) according to *FLG* mutation carrier status among 575 adult twins, showing the cumulative incidence of atopic dermatitis according to age at onset in *FLG* mutation carriers (full line) and non-carriers (dashed line) (log rank test, $P = 0.005$)

twin pairs who were discordant for AD also featured pairs who were discordant for the *FLG* mutations (Table 2).

A total of 11 DZ twin pairs discordant for an *FLG* mutation were identified. In these pairs the risk for AD was higher in the twin carrying the *FLG* mutation (five of 11 [45.5%] twins had developed AD) than in the non-carrier co-twin (two of 11 [18.2%] twins had developed AD) (OR 2.50, 95% CI 0.45–13.85; $P = 0.293$) when data were analyzed with conditional logistic regression analysis adjusted for sex and the proband's asthma status.

Based on the principles of classical twin modeling,⁸ additive genetic factors were estimated to explain 64% (33–95%; $P < 0.001$) of the susceptibility to AD and 25% (13–37%; $P < 0.001$) of the susceptibility to asthma. Including *FLG* mutation status in the classical twin models for AD and asthma, respectively, did not significantly change the estimated heritability of AD ($P = 0.328$) or asthma ($P = 0.321$).

Discussion

The present findings corroborate the suggestion that *FLG* loss-of-function mutations are substantial risk factors for the development of AD, increasing the risk two-fold.⁹ In addition, in line with previous findings,¹⁰ the association between *FLG* mutations and AD was most pronounced for persistent AD. Furthermore, use of discordant twin pairs provided a novel tool to more accurately estimate the influence of *FLG* mutation status on AD risk as this analysis inherently adjusts, to a certain extent, for several unmeasured confounding factors such as age and effects of early (pre- and perinatal) life and upbringing,¹¹ which is not possible in studies of singleton populations. However, the sample of discordant twin pairs was probably too small to fully appreciate the potential of this approach.

The study was unable to confirm the previously estimated increased risk for asthma in *FLG* mutation carriers with concomitant AD.¹ This may be because the population of twins was selected through a proband with asthma, and hence all of the twin pairs examined included at least one member with asthma. This reduced the overall variation in asthma susceptibility in the dataset and diluted the association between *FLG* and asthma, as well as the overall heritability of asthma. The same biased effect of ascertainment probably also influenced the associations found for AD because the presence of AD was associated with the selection criterion asthma. It was possible to some extent to adjust for this ascertainment bias in the analyses. However, future twin studies should ideally examine random population samples in order to fully exploit the power of the twin methodology. In such studies the definitions and grading of the severity of the AD phenotype should also be more elaborate, particularly as the natural course of AD has been shown to be highly variable.¹²

This is the first *twin* study to examine the association between atopic diseases and mutations in *FLG*. The study showed that *FLG* mutation carrier status was significantly associated with the presence and persistence of AD. Furthermore,

Table 2 Distribution of filaggrin mutations in 256 adult twin pairs according to atopic dermatitis status

	Monozygotic twin pairs ($n = 89$)						Dizygotic twin pairs ($n = 167$)					
	Concordant pairs ($n = 13$)		Discordant pairs ($n = 20$)		Unaffected pairs ($n = 56$)		Concordant pairs ($n = 11$)		Discordant pairs ($n = 57$)		Unaffected pairs ($n = 99$)	
	+AD	+AD	+AD	–AD	–AD	–AD	+AD	+AD	+AD	–AD	–AD	–AD
<i>FLG</i> mutation	2	2	4	4	4	4	2	2	11	8	10	10
Prevalence	0.15	0.15	0.10	0.10	0.07	0.07	0.18	0.18	0.20	0.14	0.10	0.10
Concordance	1.0		1.0		1.0		1.0		0.87		0.96	

Prevalence is the percentage of individuals with an *FLG* mutation. Concordance is pairwise concordance rate of *FLG* resemblance at loci R501X, 2282del4 and R2447X. Numbers are calculated from available data.

+AD, twin with atopic dermatitis; –AD, twin without atopic dermatitis.

DZ twins, like ordinary siblings, have half of their genes in common. In DZ twin pairs with an *FLG* mutation, the mutation is often carried by one twin and not by the other, and thus DZ twins represent an ideal study population for isolating the effect of *FLG* mutation status on the risk for AD. By exploiting this advantage, the current study found that DZ twin pairs discordant for *FLG* mutation status were more likely to also be discordant for AD, with the twin carrying an *FLG* null allele being the affected twin. This means that a sibling with *FLG* mutation is much more likely to have AD compared with a brother or sister who does not carry the allele.

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