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Clinical features of children with atopic dermatitis according to *filaggrin gene* variants

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Abstract
Background: **Fil**ament **agg**regating prote**in** (Filaggrin) is a skeletal cell component that provides a protective function for the epidermis. Mutations of the filaggrin gene (*FLG*) cause a loss of filaggrin protein. These mutations are seen in 50% of atopic dermatitis (AD). The aim of the study was to investigate the polymorphisms and mutations of the *FLG* in Iranian children with AD.
Materials and methods: This project was a case-controlled study with 25 children diagnosed with AD as the case group and 25 healthy children as the control group. Demographic data, clinical manifestations, and filaggrin single nucleotide polymorphisms (SNPs) and mutations were recorded. Blood samples were collected for the immunoglobulin E (IgE) assay and complete blood count tests.
Results: We found a significant association between the presence of polymorphism (rs66831674) and patients' age, and polymorphism (rs41267154) and early onset of AD. We found no significant differences between the *FLG* polymorphisms with respect to the severity of AD, ethnicity, concurrent allergic diseases, eosinophilia, and IgE serum levels.
Conclusion: Interestingly, *FLG* variants (rs66831674 and rs41267154) were associated with age and early onset of AD. However, additional studies are required to confirm these results on a large scale of Iranian population. Moreover, establishing a cohort prospective study is suggested to assess the progression of other atopic disorders based on *FLG* polymorphisms.

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Introduction

Atopic dermatitis (AD) is a common recurrent dermal disorder that occurs frequently in infants and children characterized by dryness, pruritus, and dermatitis.[1] AD occurs in patients with other atopic diseases, such as asthma, allergic rhinitis (AR), and food allergies. Infants diagnosed with AD are susceptible to allergic rhinitis and asthma. AD typically begins in infancy, and about 50% of patients show symptoms in the first year of life, and 30% of them are diagnosed at 1–5 years of age.[2]

The cause of AD is intricate and multifactorial, including genetic susceptibility, skin's barrier dysfunction, impairments in cell-mediated immunity, and immunoglobulin E (IgE)-mediated hypersensitivity.[3] Also, environmental factors, such as ultraviolet radiation (UVR), temperature, and humidity, increasing exposure to pollutants and allergens, especially mites, and decreased breastfeeding are suspected to increase the incidence of AD.[4] Loss of function mutations in the filaggrin gene (*FLG*) has led to severe AD because of loss of transepidermal water and dehydration. Moreover, additional genetic alterations have been known which may lead to epidermal barrier dysfunction and AD phenotype.[5]

Filament **agg**regating prote**in** (Filaggrin) is a skeletal cell component and a factor that causes aggregation of skeletal cells, formation of the protein–lipid matrix, and regulation of dermal permeability to water and extrinsic allergens.[6] The *FLG* is located on chromosome 1q21; the most common mutation and polymorphism are related to (p.R501X) and (c.2282del4) mutations in the European population that causes loss of function in filaggrin and predisposing factors for AD.[7] However, these European *FLG* gene mutations are rare or absent in Asian populations.[8–10]

In this study, we aimed to assess the association between Iranian young AD children's most common *FLG* variants and their characteristics such as age, sex, early onset of the disease, severity, concurrent allergic conditions as well as blood eosinophils count and IgE level.

Material and methods

This study was a reanalysis of our previous investigations performed on *FLG* polymorphisms in AD published in 2018.[11] In the stated study we identified 45 *FLG* variants. Here, we selected the most frequent *FLG* variants to investigate their association with clinical and laboratory manifestations of AD in Iranian young patients. This research was approved by the Medical Ethics Committee of Tehran University of Medical Sciences, and all patients provided written informed consent.

Study population

This research was conducted as a case-controlled study comprising 25 children aged ≤5 years with AD and referred to the Children's Medical Center in Tehran as a case group, and an equal number of healthy children as controls who had no history of AD and atopy. AD was diagnosed by an allergist based on clinical manifestations.

Questionnaire

After obtaining written informed consent, a questionnaire comprising the following was completed: demographic characteristics (age, sex, and ethnicity), clinical manifestations (age at onset of the disease, severity of AD by the scoring of atopic dermatitis [SCORAD] index, and presence of concurrent allergic conditions), and previously detected *FLG* polymorphisms and mutations.

Blood eosinophils count and total IgE serum level

Blood samples with 5 mL of blood were collected from all participants. Blood eosinophils count was detected using complete blood count (CBC) test. The total IgE serum level was determined using the human IgE Enzyme-Linked-Immunosorbent Serologic Assay (ELISA) kit (Omega; UK) according to the manufacturer's protocol.

Statistical analysis

The results were analyzed by SPSS version 16. The Kolmogorov–Smirnov test was used to define the normality of data distribution. The paired samples *t*-test was used to compare quantitative measures between different groups. Also, the Chi-square test and odds ratio (OR) with 95% confidence level (95% CI) were used to compare qualitative data; $P < 0.05$ was considered as statistically significant.

Results

In the present study, 25 children with AD comprising 17 (68%) males and 8 (32%) females were enrolled. Ten children (40%) were aged less than 6 months, 11 (44%) were aged 6–12 months, and 4 (18%) were aged more than 2 years. Early onset of the disease (at less than 6 months of age) was present in 22 patients (88%). Consanguinity was present in 8 patients (32%) and 80% of AD patients had one or more allergic comorbidities; allergic rhinitis was the most frequent concurrent allergy found in five patients (20%), followed by contact dermatitis in four patients (16%). Hot weather was the most irritant factor for AD in 16 patients (53.3%). Characteristics of the patients with AD are presented in Table 1.

IgE serum levels and blood eosinophilia were not associated with the severity of AD ($P = 0.089$ and 0.622, respectively).

The statistical analysis of the findings established that there was a significant association between polymorphism (rs66831674) and different age groups of AD patients (Table 2). However, we did not find any association between age and other polymorphisms ($P = 0.022$). Moreover, we found that polymorphism (rs41267154) was associated with early onset of AD (OR =1.50, 95% CI = 0.94–2.38), but other polymorphism did not establish any similar association with onset of the disease (Table 3).

The results demonstrated that there were no significant differences between the *FLG* polymorphisms and the severity of AD, ethnicity, and the presence of other concurrent

Table 1 Demographic and clinical characteristics of patients with atopic dermatitis.

Patients' characteristics		Number (%)
Number of AD patients		25 (100%)
Gender	Female	8 (32%)
	Male	17 (68%)
Age	<6 months	10 (40%)
	6 months to 2 years	11 (44%)
	>2 years	4 (16%)
Early onset	Positive (<6 months)	22 (88%)
	Negative (>6 months)	3 (12%)
Family history of allergic disease	No family history	5 (20%)
	Rhinitis	5 (20%)
	Urticaria	2 (8%)
	Contact dermatitis	4 (16%)
	Seasonal allergy	3 (12%)
	Food allergy	1 (4%)
	Seborrheic dermatitis and psoriasis	1 (4%)
	Rhinitis and asthma	2 (8%)
	Rhinitis and urticaria	1 (4%)
	Rhinitis and contact dermatitis	1 (4%)
Family history of atopy	No history	5 (20%)
	Parents with positive history of atopy	20 (80%)
Other allergic disease	No association	15 (60%)
	Rhinitis	4 (16%)
	Food allergy	3 (12%)
	Urticaria	1 (4%)
	Hyper-reactive airway disease	1 (4%)
	Conjunctivitis	1 (4%)
Severity of disease according to scored scale	Mild (<20)	3 (12%)
	Moderate (20–40)	11 (44%)
	Severe (>40)	11 (44%)
Immunoglobulin E (IgE)	Positive (>10)	18 (72%)
	Negative (<10)	7 (28%)
Eosinophilia	Positive (≥450)	12 (48%)
	Negative (<450)	13 (52%)

allergic diseases. In addition, there was no significant association between any *FLG* polymorphism and eosinophilia and IgE serum levels.

Discussion

Filaggrin has a significant effect on epidermal barrier; its deficiency affects the organization of cytoskeletal keratin filaments and structure of the stratum corneum (SC). Defects in *FLG* also reduce the number of keratohyalin granules, drastically reduce the concentration of natural moisturizing factor (NMF), and alkalize skin pH.[12]

Patients with *FLG* mutations have severe, early onset of, and persistent AD symptoms.[13] Contrary to these studies, we found no association between polymorphism types and severity of the disease.

In general, AD is followed by food allergy (FA), asthma, and allergic rhinitis. AD is the first presentation of atopic multimorbidity called atopic march.[13] Moreover, AD patients with *FLG* mutations present an additive risk for peanut allergy.[14] Also, in 2015, Rupnik et al. demonstrated that (2282del4) mutation was significantly correlated with allergic contact dermatitis and chronic contact dermatitis.[15] It has been established that *FLG*-related AD and asthma patients have extra hospital admissions and expenses as well as reduced health-related quality of life.[16,17] According to our cross-sectional study, no statistically significant relationship was observed between *FLG* polymorphisms and other allergies. In fact, AD is an entry point for other atopic disorders, and each of them has a dissimilar chronological development and onset time. Therefore, longitudinal prospective studies are the best study designs to identify the risk of progression of asthma and other allergies in AD patients.

Atopic dermatitis can be divided into extrinsic or allergic (increased IgE) and intrinsic or nonallergic (no increased IgE) types. Extrinsic AD is the most common type, but the incidence of intrinsic AD is about 20% and is more common in females. Extrinsic AD is strongly related to barrier disorders and Th2-related immunity, whereas reasons and mechanism of intrinsic AD have remained undetected as yet.[18] In 2007, Morar et al. demonstrated that (2282del4) and (R501x) variants were associated with extrinsic AD

Table 2 Association between *FLG* polymorphisms and age of AD patients.

Age Group *FLG* polymorphism	<6 months	6–24 months	>24 months	Total	P-value
rs11158340	5 (38.5%)	7 (53.8%)	1 (7.7%)	13 (100%)	0.410
Novel[a]	6 (40.0%)	7 (46.7%)	2 (13.3%)	15 (100%)	0.893
rs142574224	4 (44.4%)	3 (33.3%)	2 (22.2%)	9 (100%)	0.679
rs2011331	4 (44.4%)	3 (33.3%)	2 (22.2%)	9 (100%)	0.679
rs41267154	3 (33.3%)	5 (55.6%)	1 (11.1%)	9 (100%)	0.673
rs66831674	3 (27.3%)	8 (72.7%)	0 (0.0%)	11 (100%)	0.022*
Novel[b]	2 (66.7%)	1 (33.3%)	0 (0.0%)	3 (100%)	0.538
rs3126074	3 (37.5%)	5 (62.5%)	0 (0.0%)	8 (100%)	0.245

[a]In 2018, a novel polymorphism was reported in Iranian AD patients by Hasani et al.(11), with codon change of tcT>tcC and aa change of p.S417S.
[b]In 2018, a novel polymorphism was reported in Iranian AD patients by Hasani et al(11)., with codon change of Gac>Aac and aa change of p.H1961Q.

Table 3 Association between *FLG* polymorphisms and the early onset of AD disease.

Early onset *FLG* polymorphism	Yes	No	Total	P-value	OR	95% CI
rs11158340	12 (92.3%)	1 (7.7%)	13 (100%)	0.490	2.40	0.189–30.520
Novel[a]	14 (93.3%)	1 (6.7%)	15 (100%)	0.315	3.50	0.273–44.95
rs142574224	7 (77.8%)	2 (22.2%)	9 (100%)	0.238	0.233	0.018–3.026
rs2011331	7 (77.8%)	2 (22.2%)	9 (100%)	0.238	0.233	0.018–3.026
rs41267154	6 (66.7%)	3 (33.3%)	9 (100%)	0.014*	1.500	1.045–2.381
rs66831674	9 (81.8%)	2 (18.2%)	11 (100%)	0.399	0.346	0.027–0.418
Novel[b]	3 (100%)	0 (0.0%)	3 (100%)	0.495	0.864	0.732–1.020
rs3126074	8 (100%)	0 (0.0%)	8 (100%)	0.205	0.824	0.661–1.026

[a]In 2018, a novel polymorphism was reported in Iranian AD patients by Hasani et al.(11), with codon change of tcT>tcC and aa change of p.S417S.
[b]In 2018, a novel polymorphism was reported in Iranian AD patients by Hasani et al.(11), with codon change of Gac>Aac and aa change of p.H1961Q.

and increased IgE and allergic sensitivities.[19] Similarly, in 2012, Kabashima-Kub et al. demonstrated that mutations in *FLG* in IgE-high group were significantly higher than IgE-low group and healthy individuals.[20] However, we found no statistically significant relationship between common polymorphisms of *FLG* and IgE levels in AD patients.

We have revealed in this study that there is a significant association between the presence of polymorphism (rs66831674) and the age of AD patients. Moreover, our investigation on the early onset of AD disease (at an age of less than 6 months) and *FLG* polymorphisms has demonstrated a significant association between the early onset of AD and the presence of (rs41267154) polymorphism. In this regard, some studies have demonstrated a higher frequency of common *FLG* mutations in the early onset of AD in children aged less than 2 years compared with AD patients aged more than 2 years and the control group.[8,15,21]

Few studies have been conducted in Iran on AD and *FLG* mutations. The results from two studies conducted in Shiraz and Birjand revealed the AD prevalence of 1.6% and 4.3% in primary school children and those in daycare centers, respectively.[22,23] In 2006, Khaledi et al. identified six *FLG* variants in 106 Iranian AD patients and healthy controls, but no significant association was observed between these *FLG* variants and AD.[10]

However, the present research has certain limitations such as low number of selected population and not including children aged more than 5 years. Hence, additional studies with a higher sample size and covering all age groups could increase the accuracy of findings in the Iranian population.

In conclusion, we found a significant association between the presence of (rs66831674) polymorphism and age of patients, and (rs41267154) polymorphism and the early onset of AD. In addition, understanding the molecular basis of subtypes of AD and identifying suitable biomarkers could lead to establishing new targeted therapeutics and personalized medicine strategies for AD patients.

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Conflict of Interest

Authors of this research have no conflicts of interest.

Authors' Contribution

Arghavan Ziaali participated in patient care, data collection, and writing of the manuscript. Laleh Sharifi was responsible for data analysis and writing of the manuscript. Shahram Teimourian was responsible for the concept and design of the study. Bita Hasani participated in preliminary data collection and data analysis, and Anna Isaian and Mansoureh Shariat supervised the designing and execution of the study. All authors read and approved the final manuscript.

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