Filaggrin Single Nucleotide Polymorphisms in Atopic Dermatitis

Mojdeh Khaledi[1,2], Akbar Fotouhi[3], Elham Farhadi[4,5], Behnaz Mahdaviani[1,2], Soheila Sotoudeh[6], Mehdi Tavakoli[1,2], Nasrin Behniafard[5], Mohammad Gharagozlou[6], Nima Rezaei[2,4]

[1]Growth and Development Research Center, Tehran University of Medical Sciences; [2]Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences; [3]Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences; [4]Molecular Immunology Research Center; and Department of Immunology, School of Medicine, Tehran University of Medical Sciences; [5]Deparment of Hematology, School of Allied Medicine, Iran University of Medical Sciences; [6]Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

Corresponding author:
Nima Rezaei, MD, PhD
Children's Medical Center Hospital
Dr Quarib St
Keshavarz Blvd
Teheran 14194, Iran
rezaei_nima@tums.ac.ir

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SUMMARY Atopic dermatitis (AD) is a relapsing chronic pruritic inflammatory disease of skin for which no monogenic cause has been identified so far. Meanwhile, the filaggrin (FLG) gene is considered as the most important gene associated with predisposition to the disease.
One hundred and six patients with AD and 105 healthy individuals were enrolled in this study. Real time polymerase chain reaction was performed to determine frequencies of alleles and genotype in six variants of the FLG gene. The frequencies of allele A in variants of rs3126065, rs2786680, and rs1933063 as well as allele C in variant rs3814300 were 100%. There was no significant difference between allele frequencies in variants rs2485518 and rs3814299. The only genotypes in variants of rs3814299 and rs2485518 were GG and CC, respectively, with no significant difference between the patients and controls. This study demonstrated that there was no significant association between polymorphisms of FLG gene variants and AD.

KEY WORDS: atopic dermatitis, FLG, polymorphism

INTRODUCTION

Atopic dermatitis (AD) is defined as a relapsing chronic pruritic inflammatory disease of the skin which varies in location and form of the involved skin as well as different ages of infancy, childhood, and adulthood (1).

Its prevalence was reported to be 0.73% to 23% in different studies (2). While there is no countrywide study to show the exact prevalence of AD in Iran, some local studies showed a prevalence of about 15% in some areas (3). Although its prevalence varies worldwide (4), AD is a major health problem in both of developing and developed countries. It has a considerable impact on quality of life and constitutes a heavy economic burden (5). It is estimated that AD is

responsible for $364 million to $3.8 billion in expenditures in the United States annually (6).

Atopy and atopic march are strongly associated with AD (5). Atopic march refers to food allergies in infants followed by AD in first years of life and asthma, rhinitis, and other allergic disorders in later years (7).

Although worldwide variation in AD could be suggestive of the influence of environmental factors (7), considerable genetic factors were introduced as another etiology (8,9). Indeed, AD is known as a multifactorial disease (10).

Recently, Genome Wide Association Studies (GWAS) were used in order to investigate genes suspected of causing AD (5) and the whole genome was scanned in order to determine genetic variants associated with AD (11). Genes with a role in skin barrier function were primary candidates in this regard (12).

Epidermal Differentiation Complex (EDC), located on chromosome 1q21, is a group of genes involved in creation of stratum corneum as the most important component of the skin barrier. The filaggrin protein, a critical protein in this regard, is encoded by the FLG gene and located in EDC (2,7,9,10).

Filaggrin has an important role in moisturizing the skin and protecting from penetrating allergens (2,4,7). Loss of intact skin barrier, which can be due to FLG gene problems, leads to allergen penetration through the epidermal layer, which causes allergic response and immune sensitization to allergens (1,4,5,13).

Recent studies have pointed to an association between FLG mutations and AD (8), and it is now considered the most important predisposing gene for AD (14,15).

Several studies were performed to evaluate the role of Single Nucleotide Polymorphisms (SNPs), located on the FLG gene, in susceptibility to AD in different parts of the world (13,16-22). Our study was designed to investigate, for the first time, the association between 6 functional SNPs in the FLG gene and AD among the Iranian population.

METHODS

Subjects

We conducted a case control study among 106 AD patients and 105 healthy subjects as controls. All of the patients were selected from patients with Caucasian ethnic group older than 6 months who were referred to the Immunology Clinic of the Children's Medical Center Hospital, the Pediatrics Center of Excellence, Iran, and diagnosed with AD according to Hanifin and Rajka's standard criteria in the Dermatology Clinic. Patients with mild AD were excluded from the study. Severity of their disease was evaluated by the Scoring of Atopic Dermatitis (SCORAD) index. The

Table 1. Frequencies of alleles and genotypes in different FLG positions in patients with atopic dermatitis and controls

Position	Alleles	Genotype	Patients (n=106)	Controls (n=105)
			number	number
rs2485518	C		212	210
	G		-	-
		CC	106	105
rs3126065	A		212	210
	G		-	-
		AA	106	105
rs2786680	A		212	210
	T		-	-
		AA	106	105
rs3814300	C		212	210
	T		-	-
		CC	106	105
rs3814299	G		212	210
	A		-	-
		GG	106	105
rs1933061	A		212	210
	T		-	-
		AA	106	105

eczematous skin with pruritis and discharge in the most affected areas of the body, depending on the patient's age, was the phenotype of all patients with severe AD.

The control group was selected from healthy individuals without history of atopy who volunteered for this study. We attempted to select control subjects who were free from any kind of clinical atopy, such as rhinitis, asthma, and food allergy. Subjects with personal or familial history of atopy were excluded from the study as well.

This study was approved by the Ethics Committee of Tehran University of Medical Sciences. Written informed consent was signed by the parents of all enrolled patients.

Genetic study

Peripheral venous blood sampling was done for all the enrolled subjects. Real time polymerase chain reaction (PCR) was performed to determine frequencies of alleles and genotypes in six variants of the FLG gene, namely rs2485518, rs1933061, rs3126065, rs2786680, rs3814300, and rs3814299. These variants were selected based on the aforementioned polymorphisms in the FLG gene identified by research done in other countries. The aim in this study was to determine whether the same results would be found in Iranian patients.

Statistical Analysis

Allele frequencies of each variant in both patient and control groups were determined by direct counting. The odds ratio (OR) and 95% confidence interval (CI) were calculated for each allele and genotype. Chi square test was used to compare differences between the two groups. A *P* value less than 5% was considered significant.

RESULTS

As shown in table 1, the frequency of allele A in variant rs3126065 was 100% in both of patients and controls; none of the individuals had a G allele in this variant. Allele A in variants rs2786680 and rs1933063 and allele C in variant rs3814300 had frequencies of 100% as well.

The only genotype in variant rs3814299 was GG in both patients and controls. Also, in variant of rs2485518, the CC genotype was the dominant genotype among both groups.

There was no significant difference in genotype frequency in variants rs3814299 and rs2485518.

DISCUSSION

Genetic factors for allergic diseases in the literature could be categorized into 4 groups. Group 1 consists of parts of the innate immune system rolled in response to environmental exposures; group 2 includes genes related to skin barrier function; group 3 consists of factors affecting the adaptive immune system; and finally group 4 is comprised of factors modifying the tissue response to inflammation (8).

Although most prior studies focused on the innate and adaptive immune systems, recent studies were directed at genes related to epidermal barrier dysfunction in order to evaluate the pathophysiology of the disease (23).

Skin barrier dysfunction is considered one of the most important etiologies of allergic diseases, including AD. Stratum corneum and its tight junctions in the epidermal layer of the skin have an important role in acting as skin barrier.

Loricrin, involucrin, S100 gene family, small proline-rich proteins, peptidoglycan proteins, and filaggrin are some of the proteins coded in the EDC locus on chromosome 1q216, and their problems are associated with skin barrier dysfunction. FLG has been widely studied in literature in this regard (12).

Palmer *et al.* (16) reported for the first time that impaired epidermal barrier due to mutation on the FLG gene could predispose patients to AD.

The study done by Sugiura *et al.*, (24) who evaluated some gene expressions in skin biopsy specimens of patients with, revealed down regulation of FLG mRNA in patients with AD compared to the controls.

Many of these studies are restricted to studying those two null mutations of R501X (rs61816761) and 2282del4 investigated by Palmer *et al.*, but full sequencing of FLG has demonstrated polymorphisms with different frequencies in different races (12).

These mutations have been shown to predispose the Caucasian population to AD; however, these mutations were not found in non-European populations such as Asian or African ethnic groups (2). This is in agreement with the findings of the study by Nomura *et al.* (25) who found two novel mutations in Japanese patients, 3321delA and S2554X, rather than R501X and 2282del4. Winge *et al.* (26) conducted a study among Ethiopian patients with AD, in which the FLG mutation of R501X, 2282del4, S3247X, R2447X was not seen.

CONCLUSION

Our study showed that all of the patients enrolled in this study had the same allele in variants rs1933061,

rs3126065, rs2786680, rs3814300, rs2485518, and rs3814299. Our findings regarding the variants listed suggest that these variants should not be considered SNPs among the Iranian population. However, more studies with a larger sample size could increase the power of this result.

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