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The Determination of Filaggrin Gene Single Nucleotides Polymorphisms in Patients with Atopic Dermatitis

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Objectives: The protein, filaggrin, is important in barrier function and epidermal differentiation facilitation. Filaggrin gene *(FLG)* mutations have been identified as the cause of ichthyosis vulgaris (IV), and certain mutations have been associated with atopic dermatitis (AD). We aimed to investigate genetic polymorphism of *FLG* in Mongolian AD patients. **Methods:** *FLG* mutations were determined using sequence analysis in 46 AD patients and 12 IV patients. Severity of AD was assessed using the Scoring Atopic Dermatitis (SCORAD) index. Allergen specific IgE were determined from serum. Filaggrin expression in skin punch biopsy samples of AD patients was investigated using immunohistochemistry (IHC). **Results:** Several single nucleotides polymorphisms (SNPs) (1150C>T, 1741A>T, 1791C>T, 2181C>G, 2191A>G, and 2263G>A) were demonstrated in AD patients using sequence analysis. Total IgE levels were significantly associated with age (p=0.03) and duration of disease (p=0.02). Presence of SNPs and mixed allergen specific IgE was significantly correlated (p=0.02); 2 SNPs were significantly associated with food allergen specific IgE levels (p=0.009). 2263G>A SNP was significantly correlated with food allergen specific IgE (p=0.003) and a history of atopic diseases (p=0.03). **Conclusion:** New mutations or genetic polymorphisms with ethnic characteristics may be detected among Mongolians.

Keywords: Atopic Dermatitis, Filaggrin, Polymorphism, Ichthyosis

Introduction

Filaggrin is a key, multi functional protein that facilitates terminal differentiation of the epidermis and the formation of the skin barrier (keratin filaments), hence its name (FILamentAGGregationproteIN) [1, 2]. Mutations at any site within the *FLG* cause significant decreases in amounts of profilaggrin/filaggrin peptide in the skin. Loss-of-function mutations in *FLG* were the first mutation shown to be cause of the ichthyosis vulgaris (IV; Online Mendelian Inheritance in Man (OMIM) #146700). In cases of IV, marked reduction of filaggrin in epidermal keratinocyte was identified due to the nonsense mutation R501X and the frame shift mutation 2282del4 [1, 2]. Heterozygous patients with one of these mutations may have minor ichthyosis or no phenotype change, while homozygous mutations and heterozygous combinations (heterozygous for the two mutations) show marked clinical manifestations of IV with a histological defect in the skin barrier.

Atopic dermatitis (AD) is a chronic, relapsing, pruritic, and inflammatory skin disease with complex genetic and immunological background. Atopic diseases can be divided into two distinct variants: (1) the extrinsic, allergic variant, which occurs in the context of sensitization toward environmental allergens and is accompanied by elevated serum IgE levels, and (2) the intrinsic, nonallergic variant, with no detectable sensitization and with low serum IgE levels [3]. The incidence of AD in Mongolian populations has increased in the last 10 years to around 6%.

Several studies reported that approximately 25-50% of AD patients carried *FLG* mutations, a predisposing factor of AD [4]. To date, approximately 40 *FLG* mutations have been identified in IV and/or AD patients of European or Asian origin [5]. However, there are significant ethnic differences in the *FLG* mutations. In the European population, the above-mentioned two mutations (R501X and 2282del4) are prevalent, carried by ~9% of people of European origin [6]. However, these mutations are rarely found among Asian population. In Asian populations (Japanese, Chinese, Taiwanese and Koreans), 27 population specific *FLG* mutations were found [7].

The aim of our study was to determine the *FLG* polymorphism and associated clinical manifestations in Mongolian AD patients.

Materials and Methods

A total of 46 patients (34 children, 12 adults) with AD, 12 adult patients with IV, and 12 controls were enrolled in this study from January 2013 to April 2014. The AD and IV patients were from the National Dermatology Center of Mongolia. The 12 controls were healthy, adult volunteers who were sex and age matched with the 12 IV patients. The 12 controls did not have any atopic or allergic conditions and had no relationship with the other patients.

1. Ethical statement

This study was approved by the Ethical Committee Resolution (#1) of Ministry of Health Mongolia on December 29, 2012. Informed consent was obtained from each patient or their parents, and they each signed a consent form before being involved in this study. The investigator maintained confidentiality of research data.

2. Assessment of disease severity

AD cases were defined according to the diagnostic criteria Hanifin and Rajka, and the severity of AD was assessed by the same dermatologist using the Scoring Atopic Dermatitis (SCORAD) index [8]. The parameters were: (I) extent of AD, (II) intensity of skin lesions, and (III) subjective symptoms such as pruritus and sleep loss. Patients were grouped into low, mild, and severe disease, according to SCORAD values, ranging between 0 and 25, 26 and 50, or >51, respectively [9, 10].

3. Laboratory methods

Peripheral blood samples were collected, and the serum was separated. Total IgE level were measured with enzyme-linked immunosorbent assay (ELISA) (UC IgE EIA kit, Eucardio Laboratory, USA). The qualitative immunoblotting assay technique was used to measure allergen specific IgE level (Rida^{R} Allergy Screen kit, R-Biopharm, Germany).

For the molecular analysis, genomic deoxyribonucleic acid (DNA) was obtained from the whole blood of each individual and extracted by a standard procedure using the QIAampblood kit (QUAGEN, Germany). Genotyping of the *FLG* polymorphism was carried out by a polymerase chain reaction (PCR) and

sequence analysis. R501X and 2282del4 primers, designed to amplify the respective segments of interest from exon 3 of the *FLG*, were used in the amplification process (Table 1) [11]. Amplified products were purified using a multiscreen filter plate (Millipore Corp., USA), and the 312 bp and 811 bp fragments were subjected to sequence analysis (Macrogen Inc, GeumCheon-Gu, Seoul, Republic of Korea) using Big Dye (R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and ABI PRISM 3730XL Analyzer. Sequences were compared with the normal sequences of *FLG*. The sequence data allowed us to detect new single nucleotides polymorphisms (SNPs), in addition to aforementioned two null mutations. The Genbank accession number for the reference genomic sequence used for *FLG* was NG_016190.1.

Table 1. Sequences (5′-3′) of Primers Used for Genotyping and PCR[a]

Name	Product size	Forward primer	Reverse primer
R501X (57°C)	312 bp[b]	CACGGAAAGGCTGGGCTGA	ACCTGAGTGTCCAGACCTATT
2282del4 (57°C)	811 bp	AATAGGTCTGGACACTCAGGT	GGGAGGACTCAGACTGTTT

[a] PCR – polymerase chain reaction; [b] bp – base pair

Punch biopsies were performed by a certified clinical pathologist, and 5-mm samples of lesions and normal skin was obtained from adult patients. The skin samples were immediately used for immunohistochemical (IHC) studies.

Expression of filaggrin in skin was compared with age- and gender- matched controls using the IHC method (Bio Care LLC, USA). From AD patients, punch biopsies from lesional skin were collected, and from the control group, non-lesional skin from comparable areas were collected. A mouse monoclonal antibody against human filaggrin was used for staining (Abcam, Cambridge, UK).

4. Statistical analysis

Descriptive bivariate and multivariate analyses were performed using SPSS 23.0 for Windows (Chicago, Illinois, USA). For comparison, the paired Student's t-test was used for quantitative variables following normal distribution, and the chi-square test was used for qualitative variables. A non-parametric test such as Kruskall–Wallis was applied for non-normal distributions. All p-values were two-tailed, and differences were considered significant when the p-value was less than or equal to 0.05. Summary data is expressed as mean ± SD.

The sequence analysis was performed using the Chromas Lite version 2.01 (Technelysium Pty Ltd, Brisbane, Australia), DNA Baser (http://www.dnabaser.com), OMIM® (http://www.ncbi.nlm.nih.gov/omim), and db SNP programs of the NCBI (http://www.ncbi.nlm.nih.gov/snp). Population Hardy-Weinberg equilibrium (HWE) was evaluated using a χ2test, as implemented in the OEGE online tools - Chi-sq Hardy-Weinberg equilibrium test calculator for biallelic markers (Online Encyclopedia for Genetic Epidemiology Studies, http://www.oege.org/software/hardy-weinberg.html). None of the p-values presented were corrected.

Results

A total of 46 patients (34 children, 12 adults) with AD, 12 adult patients with IV, and 12 healthy adults were enrolled in this study. The mean age patients with AD were 13.8±10.1 years (children 8.7; adults 28.1). A majority (69.6%) of patients with AD were female. Demographic characteristics of participants are shown in Table 2.

Table 2. Summary of Demographic Details

	Children (n=34)	Adults (n=12)	Total (n=46)
Mean age (M±SD)[a]	8.7±4.2	28.1±7.7	13.8±10.1
Age range	3-16	20-44	3-44
Sex ratio (n (%))[b]:			
Male	12 (35.2)	2 (16.6)	14 (30.4)
Female	22 (64.7)	10 (83.3)	32 (69.6)

[a]Mean±SD – Mean±Standard Deviation; [b]n (%) –numeral (percent)

In 60.9% of AD cases, there was severe clinical manifestations. The mean duration of AD was 8.39±7.24 years. As shown in the Table 3, age of onset was significantly different between adults and children. Interestingly, the onset for a majority (41.1%) of children was between birth and first 6 months, whereas the onset for 66.6% of adults was 5 years and older (p=0.02). Also, the duration of the disease was significantly different between the children and adults (p=0.001).

Eighteen (52.9%) children out of 34 and 5 (41.6%) adults out of 12 demonstrated elevated (>100 IU/mL) serum IgE level, according to the recommendation of the Association Diseases of Allergy (ADA). Subjects in the control group did not show elevated IgE (Table 3). Correlation analysis revealed that total IgE levels were significantly associated with age (r=0.3, p=0.03) and the duration of disease (r=0.32, p=0.02).

Moreover, allergen specific IgE from the peripheral blood in

Table 3. Medical history of patients with AD[a]

	Children (n=34)	Adults (n=12)	p-value
Age (year) at onset (Mean±SD)[b]	3.1±3.8	12.5±11.9	
Duration (year) of disease (Mean±SD)	5.8±4.6	15.6±8.4	0.001***
Course of disease (n(%))[c]:			
Between birth and 6 months	14 (41.1)	-	0.02*
Between 6 – 12 months	1 (2.9)	-	
Between 1 – 2 years old	4 (11.7)	1 (8.3)	
Between 2 – 5 years old	7 (20.5)	3 (25)	
Older than 5 years old	8 (23.5)	8 (66.6)	
SCORAD[d] score (Mean±SD)	54.7±17.3	46.6±19	0.21
Severity groups (n(%)):			
Mild	3 (8.8)	2 (16.6)	0.38
Moderate	9 (26.4)	4 (33.3)	
Severe	22 (64.7)	6 (50)	
Family allergy/atopy history (n(%))			
Yes	25 (73.5)	10(83.3)	0.58
No	6 (17.6)	2 (16.6)	
Do not khow	3 (8.8)	-	
Serum total IgE (Mean±SD)	140.1±127.5	283.7±336.4	0.17
Aeroallergen sIgE[e] (n(%)):			
Yes	11 (32.3)	9 (75)	0.01**
No	23 (67.6)	3 (25)	
Food allergen sIgE (n(%)):			
Yes	11 (32.3)	5 (41.6)	0.58
No	23 (67.6)	7 (58.3)	
Mixed (aero+food) allergen sIgE (n(%)):			
Yes	4 (11.7)	3 (25)	0.36
No	30 (88.2)	9 (75)	

[a]AD- Atopic dermatitis; [b]Mean±SD – Mean±Standard Deviation; [c]n (%) –numeral (percent); [d]SCORAD – Disease severity (by score): mild <25; moderate 26-50; severe >51; [e]sIgE – specific Immunoglobulin E

* Comparison is significant at the 0.01 level

** Comparison is significant at the 0.05 level

*** Comparison is significant at the 0.001 level

patients with AD was determined. A majority (67.6%) of children were positive for aeroallergen specific IgE, whereas a majority (75%) of adults were negative for aeroallergen specific IgE (p=0.01). Serum total IgE was correlated with the severity index (r=0.28, p<0.05) in both groups. Correlation analysis revealed that food allergen specific IgE were significantly associated with a history of atopy and allergy (r=0.4, p=0.005), whereas aeroallergen specific IgE levels were significantly associated with age (r=0.37, p=0.01) in adults and children with AD (Table 3).

As previously described, two null mutations (R501X and 2282del4) were screened in the sequenced fragments amplified by PCR. No single and combined heterozygote patient with

Table 4. SNPs[a] found in AD[b] patients

SNP	Reference *	Allele change	Protein change	Residue change
1150[c]c>T[d]	-	CAT⇒TAT	His –Tyr	Basic - polar
1741A[e]>T	rs145627745	ACC⇒TCC	Thr → Ser	Polar - polar
1791C>T	-	AGC⇒AGT	Silent (Ser)	-
2181C>G[f]	rs7512779	CAC⇒CAG	His – Gly	Basic - non polar
2191A>G	-	CAA⇒CAG	Silent (Gln)	-
2263G>A	rs74129461	GAA⇒AAA	Glu – Lys	Acidic - basic

[a]SNP – Single Nucleotides Polymorphism; [b]AD – Atopic Dermatitis; [c]C – cytosine; [d]T – thymine; [e]A – adenine; [f]G – guanine;
*Obtained from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/)

Table 5. The Frequency of SNPs[a] in AD[b] patients (n (%))[c]

SNP	Total (n=46)	Age		Gender		Family history (n=21)	Severe stage (n=28)
		Children (n=34)	Adult (n=12)	Male (n=14)	Female (n=32)		
				1150C>T			
CC[d]	12 (26.1)	8 (23.5)	4 (33.3)	2 (14.3)	10 (31.4)	7 (33.3)	6 (21.4)
TC	18 (39.1)	13 (38.2)	5 (41.7)	9 (64.3)	9 (28.1)	10 (47.6)	13 (46.4)
TT[e]	16 (34.8)	13 (38.2)	3 (25.0)	3 (21.4)	13 (40.6)	4 (19.0)	9 (32.1)
				1741A>T			
AA[f]	35 (76.1)	34 (100)	11 (91.7)	14 (100)	31 (96.9)	20 (95.2)	18 (64.4)
AT	1 (2.2)	-	1 (8.3)	-	1 (3.1)	1 (4.8)	-
TT	-	-	-	-	-	-	-
				2181C>G			
CC	41 (89.1)	30 (88.2)	11 (91.7)	13 (92.9)	28 (87.5)	20 (95.2)	24 (85.7)
GC	5 (10.9)	4 (11.8)	1 (8.3)	1 (7.1)	4 (12.5)	1 (4.8)	4 (14.3)
GG[g]	-	-	-	-	-	-	-
				2263G>A			
GG	6 (13.0)	2 (5.9)	4 (33.3)	-	6 (18.8)	4 (19.0)	2 (7.1)
GA	22 (47.8)	17 (50.0)	5 (41.7)	10 (71.4)	12 (37.5)	14 (66.7)	15 (53.6)
AA	18 (39.1)	15 (44.1)	3 (25.0)	4 (28.6)	14 (43.8)	3 (14.3)	11 (39.3)

[a]SNPs – Single Nucleotides Polymorphisms; [b]AD – Atopic Dermatitis; [c]n (%) – numeral (percent); [d]C – cytosine; [e]T – thymine; [f]A – adenine; [g]G – guanine

Table 6. Allele and genotype frequencies of SNPs[a]

		1150C[b]>T[c]	1741A[d]>T	2181C>G[e]	2263G>A
		Total (n=46)			
Genotype frequency (AA/AB/BB)		12/18/16	45/1/0	41/5/0	6/22/18
Allele frequency (A/B)		0.45/0.54	0.99/0.01	0.95/0.05	0.36/0.64
	p-value	0.15	0.93	0.69	0.85
		Children (n=34)			
Genotype frequency (AA/AB/BB)		8/13/13	34/0/0	30/4/0	2/17/15
Allele frequency		0.43/0.57	1/0	0.94/0.06	0.31/0.69
	p-value	0.20	0.71	0.71	0.71
		Adult (n=12)			
Genotype frequency (AA/AB/BB)		4/5/3	11/1/0	11/1/0	4/5/3
Allele frequency		0.54/0.46	0.96/0.04	0.96/0.04	0.54/0.46
	p-value	0.57	0.88	0.88	0.57

[a]SNPs – Single Nucleotides Polymorphisms; [b]C – cytosine; [c]T – thymine; [d]A – adenine; [e]G – guanine

R501X and/or 2282del4 mutations were identified. Sequence analysis of segments of interest from exon 3 of the *FLG* revealed (rs145627745) was found in an adult patients with mild AD. The genotype frequencies of SNPs can be seen in Table 5 and 6.

Table 7. The number of multiple SNPs[a] in AD[b] patients (n[c])

	n	Children	Adult	Male	Female	Family history	Disease severity
1150TT[d]& 2263AA[e]	12	10	2	1	11	1	7
1150TT& 2263G[f]A	4	3	1	2	2	3	2
1150TC[g]& 2263AA	5	4	1	3	2	2	3
1150TC& 2263GA	13	10	3	6	7	8	10
1150TC& 2263AA& 2181GC	3	2	1	1	2	-	2
1150TT& 2263AA& 2181GC	1	1	-	-	1	-	1

[a]SNPs – Single Nucleotides Polymorphisms; [b]AD – Atopic Dermatitis; [c]n –numeral; [d]T – thymine; [e]A – adenine; [f]G – guanine; [g]C – cytosine

six SNPs (1150C>T, 1741A>T, 1791C>T, 2181C>G, 2191A>G, and 2263G>A) in patients with AD and IV (Table 4).

Among them, three novel polymorphisms (1150C>T, 1791C>T, and 2191A>G) not previously described in this genomic region were identified for the first time. These polymorphisms were located in the exon region. SNP 1150C>T and 2181C>G were generating changes in the encoded amino acid (Table 4). The newly found SNPs 1791C>T and 2191A>G were generating silent mutations.

A total of 42 patients with a history of AD were carriers of at least one of the 6 SNPs studied. Four patients were not carriers of any of these 6 SNP. The previously described SNP 1741A>T

As shown in the Table 7, 34 subjects carried more than one of the 4 SNPs that cause missense mutation. Of the four patients with three combined SNPs, three were children. Interestingly, two IV patients carried three SNPs (1150TT&2263AA& 2181G/C) (Table 6).

The previously known SNP 2263G>A was identified in 34 (73.9%) patients with AD, including 18 heterozygote and 16 homozygote cases (Figure 1). SNP 2263G>A was significantly correlated with food allergen specific IgE (r=0.42, p=0.003) and a history of atopy and allergy (r=0.31, p=0.03).

We demonstrated the filaggrin in adult patients with AD and healthy subjects using IHC. Compared to the control,

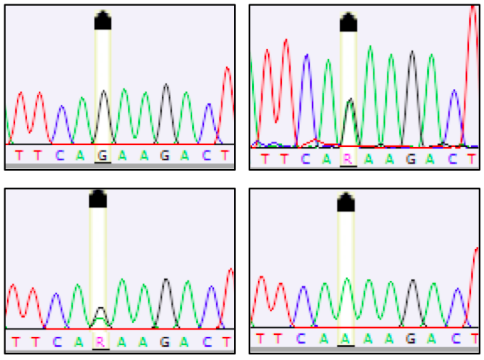

Figure 1. SNP 2263G>A in Mongolian AD patients. Each quadrant was assigned to represent normal and mutant alleles. This mutation causes missense codon (GAA ⇒AAA). Original magnification ×200.

filaggrin expression of protein levels was reduced in the epidermis of patients with 2263G>A (heter). Images of IHC and *FLG* sequence of a control (A, B) and an AD patient (C, D) are shown in Figure 2 as an example. The patient was an adult female with severe AD. The boxes in the top left corner in A and C shows the IHC results, and the arrows in B and D shows the *FLG* c.2263G>A mutation negative sequence and the *FLG* c.2263G>A heterozygote mutation, respectively. The IHC assay revealed a strong expression of filaggrin in control (A) and a decreased staining intensity of *FLG* in c.2263G>A SNP positive patient (C).

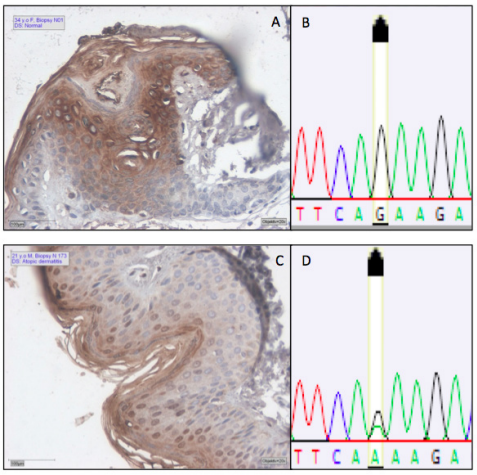

Figure 2. *FLG* mutation analysis and comparison of filaggrin expression. Original magnification ×200.

Presence of at least one SNP and mixed (food and aero) allergen specific IgE is significantly correlated. Additionally, the presence of two SNPs is significantly associated with food and mixed allergen specific IgE in patients with AD (Table 8).

Table 8. Correlation between SNPs[a] and clinical manifestations of patients with AD[b]

	Pearson correlation coefficient	p-value
Age (year) at onset	2.271	0.321
Duration (year) of disease	2.459	0.292
SCORAD[c] score	1.679	0.432
Disease severity	0.802	0.591
Serum total IgE	0.58	0.748
Aero allergen sIgE[d]	0.001	0.978
Food allergen sIgE	8.309	0.004**
Mixed (aero+food) allergen sIgE	4.888	0.027*

[a] SNPs – Single Nucleotides Polymorphisms; [b]AD Atopic dermatitis; [c]SCORAD – Disease severity (by score): mild <25; moderate 26-50; severe >51; [d]sIgE – specific Immunoglobulin E
* Comparison is significant at the 0.01 level
** Comparison is significant at the 0.05 level

Correlation analysis using a non parametric test revealed that SNPs were significantly associated with food allergen specific IgE (r=8.309, p=0.004) and mixed allergen specific IgE (r=4.888, p=0.027). Two SNPs were significantly associated with food allergen specific IgE (r=9.509, p=0.009) in patients with AD.

Discussion

AD is multi-causative disease that results from complex interactions between genetic and environmental factors. Moreover, in previously reported studies, there are obvious differences in AD between ethnic groups. If the mechanism of eczema is unclear, there is an increased acceptance of genetically determined skin barrier defect in the pathogenesis of atopy [12, 13].

Most patients (60.9%) of our study suffered from severe AD. Fifty percent of patients presented with pathological value of serum total IgE, similar with previous studies [14, 15]. Correlation analysis revealed that serum total IgE levels were significantly associated with age (p=0.03) and duration of disease (p=0.02) in patients with AD.

It is well known that AD is related to an increased production of IgE, though normal serum IgE levels do not necessarily mean that atopy does not exist. The production of IgE from B cells depend on cytokines produced by T cell and is mainly correlated with IL-4. Therefore, an important finding of our study is that

serum total IgE levels in patients were significantly higher in patients in the severe group compared to those in the mild group. This correlation depend on other factors, such as gender, age, environmental exposure to allergens. It could also depend on positive family background for atopic diseases or the coexistence of other allergic illnesses (e.g. asthma and patients), but patients with these two influencing factors were excluded from our study.

Källström et al., Laske and Niggemann, and other researchers concluded that IgE is related to the severity of eczema [16, 17]. One recent study showed that 66% of children with mild to moderate AD did not have measurable allergen specific IgE in their serum, but total serum IgE levels were significantly associated with the severity of AD [15]. The increasing levels of IgE can definitely be interpreted as from the increasing severity, as greater damage of the skin can easily lead to an increased danger of transepidermal sensitization from more allergens.

Loss-of-function mutations in the gene encoding *FLG* are the strongest and most widely replicated risk factor for the disease, suggesting a different point of view on AD pathophysiology because filaggrin is an essential component of the stratum corneum [1, 18, 19]. Two null mutations in the *FLG* gene, R501X and 2282del4, have shown great importance in IV and AD among the European population, but no clear relationship to these mutations has been determined among the Asian population [1, 7, 18, 19]. In this study, the R501X and 2282del4 mutations were not identified among Mongolian patients with AD. It is noteworthy that the allele frequencies of these mutations in our population might be lower than those obtained in European population studies. In addition, the lack of could be due to our inclusion criteria and small sample size, but it may also indicate genetic particularities of the Mongolian population.

In this study, we identified three polymorphisms in the *FLG* (1150C>T, 1791C>T and 2191A>G) that have not been previously described, and we found that two of these three results in silent mutations.

In addition to these three, we found SNP 1741A>T (rs145627745) in an adult patient with AD, as Cubero et al. previously found in the Spanish population. SNP 1741A>T showed an association with non-allergic, severe asthma. This is different than some mutations in the *FLG* which have been associated with the presence of asthma (in particular, allergic asthma and asthma with AD). Despite having been described in the above mentioned data, SNP 1741A>T has not been studied in populations of patients with AD [20].

Another SNP we found was 2263G>A (rs74129461), which has interestingly been found in Korean AD patients [21, 22]. The genotype frequencies of SNP can be seen in Table 5, 6. It is found in genomic position 2263 and is a guanine-to-adenine transition. The protein carries a modification in position 755, where the amino acid glutamic acid (Glu) changes to a lysine (Lys), so this SNP can also be designated as p.E755K orp. Glu755Lys. This change was present in 40 subjects (32 children and 8 adults) (Table 5). The genotype and allele frequencies were similar with findings from Korean IV and AD patients. No statistically significant differences were found when analyzing age or gender between Mongolian and Korean patients.

2263G>A SNP was significantly correlated with food allergen specific IgE (p=0.003) and a history of atopy and allergy (p=0.03). SNP and mixed (food and aero) allergen specific IgE were significantly correlated (p=0.02). Two SNPs were significantly associated with food allergen specific IgE (p=0.009) in adults and children. Interestingly, SNPs and AD severity did not correlate. This could be due to the inclusion criteria and small sample size of the study, but it may also indicate particular genetic aspects of the Mongolian population.

FLG is very difficult to analyze because of its large size (>12 kb) and highly repetitive nature, but previous studies have demonstrated significant results using smaller sample sizes [2, 23, 24]. Our study was a small scale study investigating a particular region of interest to determine the presence two known null mutations of IV and AD (R501X and 2282del4). In our population, we found no significant prevalence of the null mutations, R501X and 2282del4, in AD patients, possibly due to following limitations of our study: 1) relatively small sample size; 2) a study designed to investigate only the region of interest of *FLG*. Our preliminary study reveals the need and basis for a comprehensive study to determine genetic predisposition to AD among Mongolians. Therefore, future study will focus on increasing sample size and performing whole gene sequencing of *FLG*.

In addition, our study identified three polymorphisms that have not been previously described in *FLG* (1150C>T, 1791C>T and 2191A>G). The SNPs (1150T>C, 2263G>A) seems to be more associated with AD in Mongolians, though further study on this topic is necessary. We hope to extend this study on

Mongolian patients with AD and, in future studies, compare the genetic diversity of *FLG* of Mongolians with other ethnic groups.

In conclusion, to the best of our knowledge, this is the first report describing *FLG* polymorphism in Mongolian patients with AD. Our study shows that new mutations or genetic polymorphisms with ethnic characteristics need to detected among Mongolians, and and larger studies of repeat number polymorphisms should be performed in the future.

Conflict of Interest

The authors state no conflict of interest.

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