

Filaggrin Mutations in a Western Siberian Population and Their Association with Atopic Dermatitis in Children

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We determined the frequencies of null mutations of the *FLG* gene—2282del4, R501X, R2447X, 3702delG, S3247X, and the 12-repeat allele (rs12730241)—among 460 Caucasians of the city of Novosibirsk, Russia. The frequency was 17.7% for rs12730241, 2.73% for 2282del4, 0.22% for R501X, 0.33% for R2447X, and 0% for 3702delG and S3247X in a western Siberian population. A case–control study showed that the deletion 2282del4 was associated with atopic dermatitis in children (odds ratio 7.01; $p < 0.001$). The other mutations were not.

Introduction

THE *FLG* GENE (filaggrin: filament-aggregating protein) is located in the gene cluster of the epidermal differentiation complex at 1q21.3 and consists of three exons. The third exon is the longest (12.7–14.7 kbp) and contains tandem repeats (10–12) totaling 972 bp and encoding 324 amino acid residues (Gan *et al.*, 1990).

Human *FLG* encodes profilaggrin, a multimeric precursor >400 kDa, which is a highly phosphorylated histidine-rich protein (Ovaere *et al.*, 2009; Osawa *et al.*, 2011). During terminal differentiation of keratinocytes, profilaggrin is dephosphorylated and disintegrates into 10–12 monomers ~37 kDa and a proteolytically cleaved N-terminal domain containing the S100 calcium-binding motif that participates in the regulation of profilaggrin processing (Fleckman *et al.*, 1985; Markova *et al.*, 1993; Presland *et al.*, 1992; Smith *et al.*, 2006). The resulting monomers are transformed into components of the natural moisturizing factor (NMF) in the skin (Mildner *et al.*, 2010; O'Regan *et al.*, 2010; Chen *et al.*, 2011; Hsu *et al.*, 2011).

Studies of the relationship of the profilaggrin level with skin diseases started in the 1980s (Dale *et al.*, 1985; Fleckman *et al.*, 1985; Palmer *et al.*, 2006; Smith *et al.*, 2006; Compton *et al.*, 2002). At present, it is known that one of the reasons of the low concentration of profilaggrin in the granular layer of the skin is null mutations of *FLG*, which produce premature stop codons and result in an inactive protein; this problem leads to formation of an aberrantly thin layer of keratinocytes, thinning of the protective layer of the skin, downregulation of components of NMF, transdermal loss of water, and excessive sloughing of the skin (Smith *et al.*, 2006; Kezic *et al.*, 2008). The disruption of integrity of the protective layer makes the skin permeable to

pathogens, xenobiotics, and allergens, thereby increasing the risk of allergic diseases (Cork *et al.*, 2009; Rodriguez *et al.*, 2009; Jungersted *et al.*, 2010; Osawa *et al.*, 2011). Numerous case–control studies and familial analyses show that null mutations of *FLG* are risk factors for atopic dermatitis (AD) (Rodriguez *et al.*, 2009).

Among patients with ichthyosis vulgaris and AD (separately) more than 40 null mutations of *FLG* have been uncovered in Asian and Caucasian populations (O'Regan *et al.*, 2010; Akiyama, 2010; Osawa *et al.*, 2011). All null mutations make the protein nonfunctional. Depending on the genotype, namely the type and number of mutant alleles, there are various clinical manifestations and degrees of disruption of the protective layer of the skin (Palmer *et al.*, 2006; Sandilands *et al.*, 2006; Smith *et al.*, 2006; Irvine *et al.*, 2011; Osawa *et al.*, 2011).

Research has shown that the spectrum of *FLG* mutations is specific to various populations and races. For instance, the pathogenesis of AD among Asian populations involves *FLG* mutations that are different from those in Caucasians (Akiyama, 2010; Chen *et al.*, 2011). Among Asian populations, the AD-associated mutations are 3321delA, Q2417X, R501X, R4307X, 6859del8, S1515X, and some others (Akiyama, 2010; Chen *et al.*, 2011). The most frequent null mutations among populations in Europe and North America are R501X and the deletion 2282del4. The presence of these mutations is a risk factor for ichthyosis vulgaris (Smith *et al.*, 2006) and atopic diseases (Palmer *et al.*, 2006; Sandilands *et al.*, 2007). According to Irvine *et al.* (2011), the main mutations that account for the development of AD in European populations are the following: R501X, 2282del4, S3247X, 3702delG, and R2447X. R501X is implicated in 39% of AD cases, 2282del4

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DNA extraction and genotyping

DNA was isolated from the whole blood using the RealBest Extraction 100 Kit (Vector-Best). We selected the following mutations for analysis: 2282del4, R501X, R2447X, 3702delG, S3247X, and the polymorphism rs12730241 associated with the 12-repeat allele of *FLG*. The mutations were detected by means of rapid high-throughput fluorescence kits that we developed ourselves. The method is based on the polymerase chain reaction (PCR) and subsequent melting curve analysis of the complexes of amplicons with a specific probe, as described previously (Prasolova *et al.*, 2013). PCR was performed on a fluorescence cyclor CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad). To confirm the results, the DNA samples (both wild type and mutant) were sequenced at the Genomics Center of Shared Resources of the Siberian Branch of the Russian Academy of Sciences (SB RAS, Novosibirsk). Detection of R501X and 2282del4 was also performed by means of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described previously (Palmer *et al.*, 2006; Smith *et al.*, 2006).

Statistical analysis

The possible link of the mutations with AD was evaluated using an odds ratio (OR) in the EpiInfo6 software. While assessing the association of a mutation with the disease, when the number of cases was <5 we used Fisher's exact test. Comparison of frequencies between the groups was conducted by means of the χ^2 test. The differences were considered statistically significant at $p < 0.05$.

Results and Discussion

FLG genotyping

The mutations were detected as described previously (Prasolova *et al.*, 2013), and the results were verified by sequencing. Figure 1 shows sequencing data from wild-type and mutant *FLG* regions. Among the tested mutations and polymorphisms, we detected the following: 2282del4, R501X, R2447X, S3247X, and rs12730241. The deletion 3702delG was not found in any of the groups. Comparison of the results of PCR-RFLP and of analysis of melting curves (real-time PCR) for detection of 2282del4 and R501X showed that the 2 methods yielded 100% identical results.

Frequencies of FLG mutations in the study groups

The frequency of the polymorphism rs12730241 of the *FLG* gene in our study population ($n=460$) was 17.7%, which is not significantly different ($p=0.043$) from the Irish population (14.6%; Brown *et al.*, 2012).

Among the null mutations in our population sample, the deletion 2282del4 was found in >1% of the participants (frequency >1%), and the mutations R501X, R2447X, and S3247X each had a frequency <1%, whereas carriers of 3702delG were not found (Table 1). In European countries, R501X is the most frequent, with the frequency of 1–4% among the residents of Ireland, Scotland, Germany, and Poland and among North American Caucasians (Palmer *et al.*, 2006; Smith *et al.*, 2006; Sandilands *et al.*, 2007; Weidinger *et al.*, 2007; Huffmeier *et al.*, 2008; Poninska *et al.*, 2011; Brown *et al.*, 2012), whereas in our study sample, the

TABLE 1. DISTRIBUTION OF FREQUENCIES OF MUTATIONS IN THE *FLG* GENE IN DIFFERENT CAUCASIAN POPULATIONS

Mutation	Mutant (minor) allele frequency, %					
	Novosibirsk, Russia (our present results)	Ireland	Germany	Scotland	Caucasians in North America (Smith <i>et al.</i> , 2006)	Poland (Poninska <i>et al.</i> , 2011)
R501X	0.22 (460)	4.1* (97) (Smith <i>et al.</i> , 2006) 3.17* (189) (Palmer <i>et al.</i> , 2006) 1.3** (736) (Sandilands <i>et al.</i> , 2007)	1.8** (367) (Huffmeier <i>et al.</i> , 2008) 2.4** (251) (Weidinger <i>et al.</i> , 2007)	2.1* (145) (Smith <i>et al.</i> , 2006)	2.4** (124)	1 (510)
2282delCAGT	2.73 (460)	0.5 (91) (Smith <i>et al.</i> , 2006) 1.1 (186) (Palmer <i>et al.</i> , 2006) 1.3 (736) (Sandilands <i>et al.</i> , 2007)	2.8 (362) (Huffmeier <i>et al.</i> , 2008) 1.8 (250) (Weidinger <i>et al.</i> , 2007)	1.2 (166) (Smith <i>et al.</i> , 2006)	1.1 (133)	3.79 (510)
R2447X	0.33 (460)	0.13 (736) (Sandilands <i>et al.</i> , 2007)	—	—	—	—
S3247X	0 (460)	1.1 (736) (Sandilands <i>et al.</i> , 2007)	—	—	—	—
3702delG	0 (460)	0.01 (736) (Sandilands <i>et al.</i> , 2007)	0 (372) (Huffmeier <i>et al.</i> , 2008)	0 (195) (Sandilands <i>et al.</i> , 2006)	—	—
rs12730241 (12-repeat allele)	17.7 (460)	14.6 (928) (Brown <i>et al.</i> , 2012)	—	—	—	—

The sample size, that is, the number of people, and abbreviated literary references are shown in parentheses; ** $p < 0.01$, * $p < 0.001$.

TABLE 2. THE FREQUENCY OF MUTATIONS IN *FLG* AND THEIR ASSOCIATION WITH ATOPIC DERMATITIS

Mutation/polymorphism	Frequency,% (number of mutant alleles)		Odds ratio (95% confidence interval)	p-Value
	Nonatopic control	AD		
2282del4	1.66 (3)	11.76 (33)	7.88 (2.41–40.67)	0.00016
R501X	1.11 (2)	1.07 (3)	0.96 (0.11–11.65)	0.65
R2447X	0	0.36 (1)		
S3247X	0	0.70 (2)		
Presence of R501X, R2447X, or S3247X	1.11 (2)	2.15 (6)	1.95 (0.34–19.93)	0.332
rs12730241	18.32 (33)	14.70 (40)	0.74 (0.44–1.27)	0.246

AD, atopic dermatitis.

frequency is 0.22% ($P < 0.01$). In our sample, the deletion 2282del4 has a frequency of 2.73%, whereas in Ireland and Scotland, the frequency is lower, 0.5–1.5% (Smith *et al.*, 2006; Sandilands *et al.*, 2007), and in Germany, it is consistent with our data, 1.8–2.8%, as is the case for North American Caucasians (Palmer *et al.*, 2006; Smith *et al.*, 2006; Sandilands *et al.*, 2007; Huffmeier *et al.*, 2008). Other mutations are rare in all populations, with the frequency $< 1\%$ (Irvine *et al.*, 2011). The frequency of R2447X in our study sample is somewhat higher than in a sample from Ireland (0.33% versus 0.13%), but this difference is not statistically significant ($p = 0.32$; Table 1). In an Italian population sample, filaggrin mutations are rare and altogether amount to 1.4% (Giardina *et al.*, 2008). The frequencies of R501X and 2282del4 among Italian AD patients are 0.6% and 0.9%, respectively, and in the general population control they affect less than 0.5% of the population (Cascella *et al.*, 2011). The frequencies in our study population are in line with those among Caucasian populations of Germany and Poland (Table 1). These results confirm the literature data on the heterogeneity of frequencies of *FLG* mutations among Caucasian populations of different countries. For instance, null mutations of *FLG* are more frequent among Caucasians in the countries of northern Europe, such as Ireland and Scotland, whereas in southern Europe, in Italy, the frequency of these mutations is lower (Cascella *et al.*, 2011; Irvine *et al.*, 2011).

Association of the mutations with AD

In the group of children with AD and in the control children without a history of AD, the most frequent mutation was the deletion 2282del4; the frequency in the AD group was 11.76% and in the NC group it was 1.66% (Table 2). R501X, R2447X, and S3247X were rare mutations with frequencies $\leq 1\%$; R2447X and S3247X were undetectable in the NC group. The deletion 3702delG was not found in any group.

Our data show that the highest risk of AD is associated with 2282del4 (OR 7.88, 95% confidence interval [CI] 2.41–40.67; Table 2). We could not calculate the OR for the mutations R2447X and S3247X because the control group does not contain such carriers. Because all of the null mutations under study make the *FLG* protein nonfunctional (Smith *et al.*, 2006; Kezic *et al.*, 2008), we assessed the influence of the presence of any of the rare mutations (R501X, R2447X, or S3247X) on the risk of AD; the OR was 1.95 (95% CI 0.34–19.93).

Even though we did not obtain statistically significant results for the rare mutations of *FLG*, their biological conse-

quences, namely formation of a nonfunctional protein, suggest that these mutations are still responsible for disruption of the protective layer of the skin. A combination of such mutations has an additive effect (Smith *et al.*, 2006; Carlsen *et al.*, 2013). In a recent study, researchers conducted analysis of a chromosomal position of the mutations R501X and 2282del4 in alleles during their simultaneous presence in 37 patients (compound heterozygotes); the results showed that the mutations are in the *trans* arrangement and do not occur within the same allele (Carlsen *et al.*, 2013). Among our patients, there were no combinations of two or more mutations of *FLG*.

The polymorphism associated with the 12-repeat allele of *FLG* was more frequent in the NC group than among the AD patients (Table 2); the homozygous genotype of 12/12 repeats was four-fold more prevalent in the control group (NC) than in the AD group. Although these differences are not statistically significant, an OR of 0.74 is suggestive of the protective role of the 12-repeat allele (For statistical significance of this OR value with probability $[1 - \alpha]$ of 95% and statistical power $[1 - \beta]$ of 80%, we would need a sample size of at least 504 people in the NC group and 842 participants in the AD group). Our OR value is in agreement with the literature; namely, the carriers of the 12-repeat allele are at a lower risk of atopic diseases than are carriers of the 10-repeat allele (Brown *et al.*, 2012). Thus, in the present work, we found that the strongest statistical association with AD belongs to the deletion 2282del4. Because of the low frequency of the other mutations under study, we were not able to obtain statistically significant data on their contribution to the risk of AD.

In conclusion, the frequency of R501X is significantly lower, whereas the frequency of 2282del4 is higher among Caucasians in Novosibirsk than in other Caucasian populations. Frequencies of R2447X, S3247X, and rs12730241 in our study population are not significantly different from other Caucasian populations.

All null mutations are linked to predisposition to AD, but the polymorphism rs12730241 is associated with resistance to AD; these data are consistent with studies on other similar populations. The frequency of AD in Novosibirsk is 5.8–9.0%, depending on age (Kondyurina *et al.*, 2004); therefore, the pathogenesis of AD in the western Siberian population may involve additional defects of *FLG*, aside from those analyzed in our study.

Author Disclosure Statement

No competing financial interests exist.

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