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Possible immunological consequences of filaggrin gene mutation. A case study of a 3-year-old allergic girl

ANNA OGRODOWCZYK, BARBARA WRÓBLEWSKA, LIDIA HANNA MARKIEWICZ, MAGDALENA ZAKRZEWSKA

Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Poland
Non-Public Health Care Clinic of Allergy "ALLERGICA", Olsztyn, Poland

Abstract

Filaggrin is one of the most important structural proteins in the stratum corneum of the human's epidermis. There are known over forty mutations in the filaggrin gene (FLG) of which the null-type mutations such as R501X and 2282del4, localized in exon 3 of this gene, are the most significant risk factors for the development of allergic diseases. The atopic diseases develop in mucosal surfaces such as the gastrointestinal tract, the respiratory tract and the skin. We focus on research characterizing the filaggrin-deficient epidermis with respect to genetic, immunologic and microbiome abnormalities to show the consequences of these mutations in the development and progression of atopy. A case of a 3-year-old girl with food allergy, atopic dermatitis and Staphylococcus aureus infection, which was found by the presence of R501X mutation in the filaggrin gene. The girl remains under strict medical control and is subjected to integrated therapy of allergic diseases.

Key words: *Filaggrin mutation, food allergy, atopic dermatitis, Staphylococcus aureus infection.*

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Introduction

This paper describes a case of a 3-year-old girl with atopic dermatitis, sensitized to milk, eggs, pork meat, celery and grain flour proteins. The girl was born by caesarean section. She had neither history of allergy nor eczema in the family. During the first four weeks of life, she was exclusively breast-fed with no supplementation. In the second month of life, she experienced symptoms of atopic dermatitis that were worsened by products containing cow's milk, eggs and celery in the mother's diet. The level of total IgE in serum was determined at 468.5 kU/l. Although the child's symptoms always correlated with specific food, it was difficult for the mother to follow the elimination diet successfully due to the presence of hidden allergens. In the third month of the girl's life, she often started to vomit a few minutes after feeding and suffered from alternating constipation and diarrhea. She had also a similar reaction to Nutramigen (an extensively hydrolyzed casein) and Bebilon Pepti (an extensively hydrolyzed whey protein) enhanced with shortness of breath. She required many hospitalizations for dehydration and low body mass (weight at the 13th percentile and height at the 25th percentile). According to the SCORAD scale, 97% of her skin had been affected by the severe (3 according to 0-3 scale of intensity) signs along with oozing, abscesses and crusting, by the end of her first year of life. Laboratory data revealed infection of scarves by methicillin-resistant *Staphylococcus aureus.* After further analysis, the presence of this strain in feces, urine and saliva samples was confirmed. Beside this, in the swab from the throat and sinuses, penicillin-resistant *Streptococcus viridans* was also present. During hospitalization, a diet with an amino acid-based formula Bebilon Amino (Nutricia) and Neocate (SHS) was successfully introduced. Since then, she was on this type of diet until the age of two. During the 17th month of life there were attempts to gradually turn to the diet of bread and biscuits but symptoms such as abdominal pain and vomiting occurred. After this diet she was at the 35th percentile for

Correspondence: Anna Ogrodowczyk, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Tuwima 10, 10-748 Olsztyn, Poland, e-mail: ania.ogrodowczyk@o2.pl

weight. It is worth mentioning that the pork meat, hen's egg, celery were not included in the child's diet and her symptoms also did not always correlate with specific food. Finally, she was instructed to avoid milk, dairy products, bread and products based on wheat and corn flour, celery and chicken eggs. She was also treated with Cetirizine and Hydroxyzine and underwent a full Cefotaxime therapy. The mother of the girl currently prepares all the meals on her own and the child does not eat any readymade products. During the last year, the total serum IgE level did not decrease in spite of proposed treatment. The slow atopic dermatitis (AD) wound healing has led to looking for possible null-type mutations in the filaggrin gene (FLG) in the described patient. The increasing number of products that trigger response from the gastrointestinal tract increased interest in the patient's gut condition.

Material and methods

Blood and stool samples were obtained from a 3-year-old girl suffering from food allergy and atopic dermatitis with a previously diagnosed infection of *S. aureus.* The procedures were followed in accordance to the Helsinki Declaration of 1975, revised in 2000, and with the standards of the local ethical committee that gave the approval for conducting the research (Case No. 2/2010).

Immunological analysis

The patient's total IgE level was measured using ImmunoCAP (Phadia AB, Uppsala, Sweden), and food specific serum IgE has been determined with EUROLINE Pediatric profile for allergy diagnosis (EUROIMMUN AG, Lübeck, Germany). Tests were confirmed by the percutaneous skin tests (Allergopharma-Nexter, Germany). The patient's serum IgG and cytokine (e.g. IL-2, IL-4, INF-γ) levels have been determined by means of the enzyme linked immunosorbent assay method (ELISA) (BD Bioscience, USA). The levels of patient's secretory IgA, Calprotectin and α-1-antitripsine (A1AT) were evaluated in the stool sample also with the enzyme linked immunosorbent assay method (ELISA) (ImmunDiagnostik AG, Germany).

Molecular analysis

Genomic DNA was isolated from EDTA blood samples according to the protocols of the producer (EURx Molecular Biology Products, Gdańsk, Poland).

The analysis of the R501X (c.1501C>T) mutation of the FLG gene required genomic DNA amplification according to the Smith *et al.* 2006 protocol. The protocol: PCR mixture buffer containing 1.5 mM $MgCl_2$, Taq polymerase, primer pair FilH1F3/RPT1P6 using the Eppendorf MasterCycler® gradient (Eppendorf, Germany). PCR conditions were as follows: 94˚C for 5 min; 30 × of 94˚C/30s, 58˚C/45s, 72˚C/1 min; at the end one cycle 72˚C for 5 min. The presence of p.R501X mutation creates a specific *Hin1II* (*NlaIII* – Fermentas) restriction site. Products were digested: 5 Unit, 4 h at 37˚C and resolved in 3% high resolution agarose gel.

The analysis of the c.2282del4 mutation also required amplification with the primer pair RPT1P7/RPT2P1 and enzyme digestion. PCR conditions were as follows: 94˚C for 5 min; 35 × of 94˚C/30s, 57˚C/45s, 72˚C/1 min 30 s; at the end, one cycle 72˚C for 5 min. The presence of 2282del4 mutation creates a specific *AdeI* (*DraIII* – Fermentas) restriction site. Products were digested: 5 Unit, 4 h at 37˚C, and resolved in 2% high resolution agarose gel. Amplified PCR products were also resolved in 1.5% agarose gel, purified according to the protocols of the producer (EURx Molecular Biology Products, Gdańsk, Poland) and sequenced using Genome Sequencer Junior.

Results

Immunological analysis

The patient's total IgE and IgG serum level significantly exceeded the standard norm required for the age (Table 1). The specific serum IgE test results were markedly positive for both egg white and yolk, milk, wheat, walnut, soybean and raw potato (Table 2). The percutaneous skin test results confirmed multiple hypersensitivity (Table 3). The test result was considered positive if the wheal diameter was 3 mm or more greater than the negative control.

The levels of pro-inflammatory cytokines such as IFN-γ, IL-2 were detectable and IL-4 in the serum sample were undetectable. The profile of cytokines produced by peripheral blood cells indicate Th1 type of response. Otherwise, the increased level of IgE and IgG in serum can partially explain the lack of IL-4 and it indicates the active IgE-mediated mechanism of allergy. The level of secretory IgA in a stool sample was also high. However, it was in the normal range in contrast to calprotectin, the level of which was significantly higher than the normal range (178.2 μg/g). Cal-

Table 1. Results of serum and stool samples and the norm appropriate for the age

Type of test	Result	Normal range [test limit sensitivity]	Type of test	Result	Normal range
INF-γ	128.12 ±4.14 pg/ml	[4.7-300 pg/ml]	IgE	1107 kU/L	< 40 kU/L
IL-2	22.72 ±1.82 pg/ml	[7.8-500 pg/ml]	sIgA	1714 ±7.89 μg/g	510-2040 μg/g
IL-4	below	[7.8-500 pg/ml]	calprotectin	178.2 ±1.18 μg/g	< 166 μg/g
IgG	23.3 ±1.13 g/l	9.0-15.0 g/l	A1AT	15.98 ±2.21 mg/dl	< 26.8 mg/dl

Table 2. Food-specific serum IgE results. The normal range for all < 0.35 (kU/L)

Type of allergens	Patient range (kU/L)	Class of reaction
egg white (f1)	42	4
egg yolk (f75)	13.5	3
milk (f2)	9.59	3
α-lactoalbumin (f76)	2.5	2
β-lactoglobulin (f77)	< 0.35	0
casein (f78)	1.1	2
soybean (f14)	10.6	3
codfish (f3)	0.37	1
peanut (f13)	< 0.35	0
walnut (f17)	10.6	3
wheat (f4)	1.1	2
carrot (f31)	< 0.35	0
rice (f9)	< 0.35	0
apple (f49)	< 0.35	0
potato (f35)	60	5

protectin is named after its ability to bind calcium and zinc. It is 36.5 kDa protein expressed by the gene S100. It has bacteriostatic and mycostatic properties, is abundant in neutrophil granulocytes and plays an antimicrobial role in the defense of the organism. An increased calprotectin concentration in stool may be the consequence of neutrophil degranulation due to mucosal damage, which is why it is treated as a marker in many inflammatory bowel diseases [1, 2]. Also, the level of α_1-antitrypsin (A1AT) in the stool sample was determined. A1AT is a protease inhibitor, belonging to the serpin family, weight 52 kDa. A1AT plays a regulatory and anti-inflammatory role. Fecal A1AT is considered as a marker for the intestinal protein loss and permeability. Moreover, it can be used as a marker of inflammatory intestinal diseases [3]. The result of the test for concentration was estimated as negative, but it is worth mentioning that the result should be expanded to include an assessment of A1AT clearance-measurements.

Molecular analysis

The molecular analysis revealed the presence of R501X mutation in repeat one of exon three of FLG. The patient has been confirmed to have R501X mutation in the homozygous system. The transition in position 501C→T resulted in occurrence of null type mutation. The presence of R501X mutation was confirmed in restriction digestion with the *Nla III* enzyme, as well as during the sequence analysis. 2282del4 has not been detected by any of the used methods (Fig. 1).

Table 3. Percutaneous skin test-result

Type of allergen	Wheal (mm)	Flare (mm)
histamine control	10	7
saline control	1	1
pork meat	5	4
chicken meat	3	4
turkey meat	3	3
peanut	–	–
walnut	5	4
mandarin	–	–
lemon	–	–
orange	–	–
apple	–	–
strawberry	2	3
egg white	6	4
egg yolk	4	4
rye flour	3	2
wheat flour	6	5
corn flour	5	5
cocoa	3	3
tomato	–	–
potato	5	5
celery	7	4
pepper	–	–
codfish	2	2
milk	5	4

Discussion

Over the last few years, there has been a significant increase in prevalence of allergic diseases as well as the increased development of knowledge in the field of immunology and allergology. The treatment of patients in many cases can be hampered by a number of possible complications, such as infections but also by genetic mutations. This case was an example of a combined microbiological, immunological and genetic dysfunction. Invalid filaggrin protein in the skin barrier, plays a major role in sensitization to antigens via skin [4, 5]. Mutations in the filaggrin gene show associations with AD and can exacerbate allergic diseases [6-8]. Moreover, patients with AD may display a significant decrease in the expression of antimicrobial peptides which explains the susceptibility to secondary skin infections with pathogens [9]. It is a result of presence of the null-type filaggrin mutation and reduction in the level

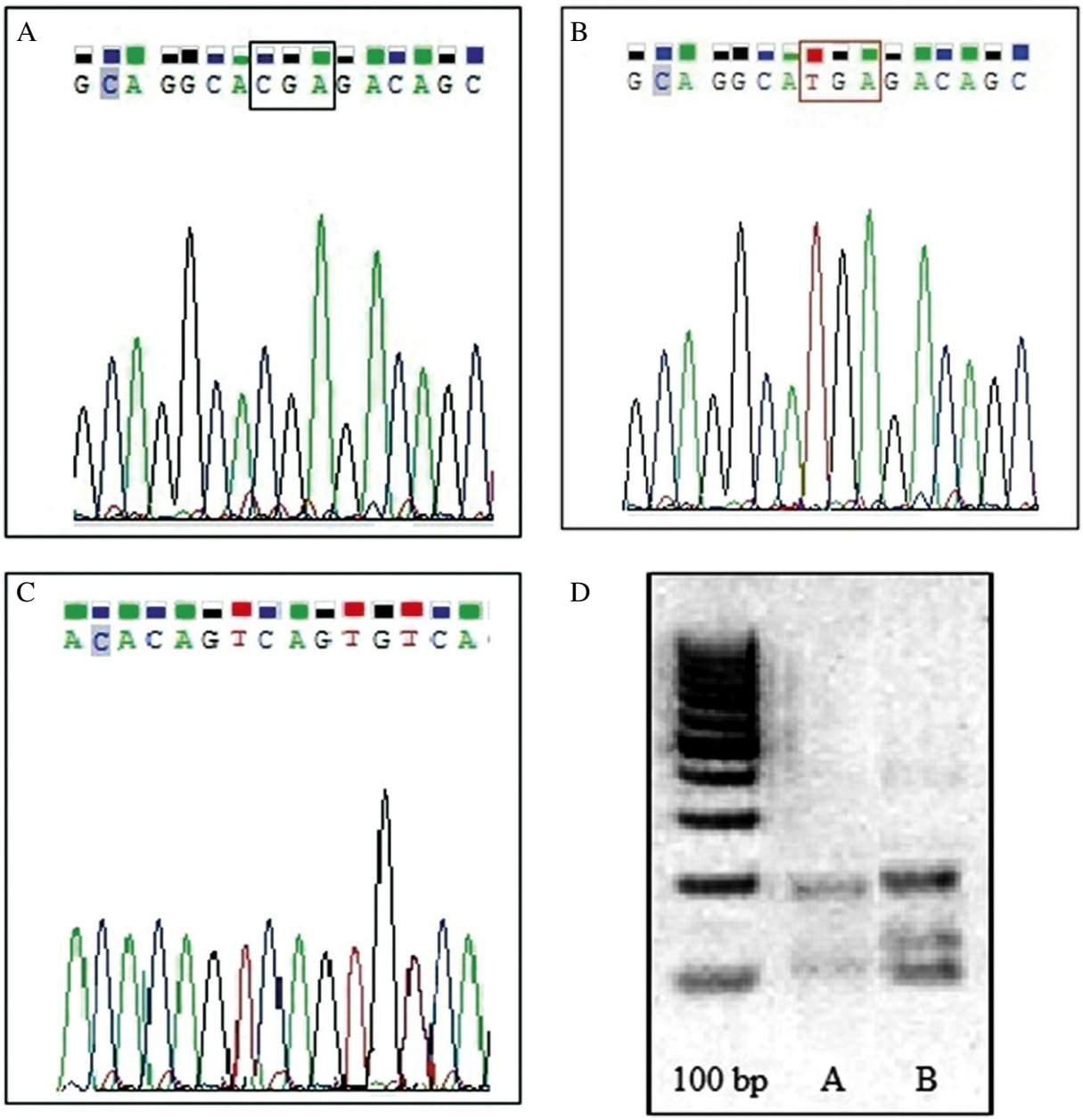

Fig. 1. Filaggrin R501X mutation detection and confirmation A) and B); the lack of 2282del 4 mutation in codon 713-717 C); R501X detection – electrophoresis D)

of filaggrin breakdown products on the skin (urocanic acid – UCA, and pyrrolidone carboxylic acid – PCA). It makes the skin more sensitive to *S. aureus* infection because of its alkalization [10, 11]. The literature data proved that more than 90% of patients with AD suffer from infection with *S. aureus* [12, 13]. In many cases, including the one described above, there is a problem with successful therapy because of the strain's resistance to various antibiotics [14]. *S. aureus* produces enterotoxins that stimulate polyclonal T cells without requiring antigen presentation. Such activity is known as the superantigen effect [15]. The production of IgE and IgG can be also stimulated by *S. aureus* protein known as fibronectin-binding proteins (FBPs). The protein is important for this strain because of the mechanism of adhesion and invasion dependent on the process of binding FBPs to fibronectin [16]. The mechanism that takes part in developing humoral and cellular immune responses is dependent on antigen presentation [17]. The stimulation with FBP results in a high release of proinflammatory cytokines (e.g. IFN-γ, IL-6, TNF-α) which may play an important role in

various inflammatory processes, including AD. The skin affected by AD is frequently colonized by α-toxin–producing strains of *S. aureus* [18]. It has been discovered that properly formed filaggrin protects keratinocytes against the lethal effects of α-toxin and plays a crucial role in protecting cells, by mediating the secretion of sphingomyelinase that reduces the number of α-toxin binding sites on the keratinocyte surface. The presence of null-mutation in filaggrin gene results the increased propensity to *S. aureus* infection [19]. Although there is no expression of filaggrin gene in the intestinal epithelium or distal, mid- or proximal esophageal epithelium but the presence of mutation in the genome succeed an increased immunological systemic response [20]. Systemic reaction can increase permeability of intestinal epithelium and can intensify penetration of antigens through the intestinal mucosa that can be an effect of the inflammation state of intestine epithelium. This in turn can lead to abdominal pain, vomiting, nausea and chronic diarrhea [21].

The described case of a 3-year-old girl indicates the need of a multifaceted diagnosis and comprehensive treatment of patients with allergic diseases. Proper diagnosis and early introduced therapy can help avoid complications. The proper care of atopic skin is crucial and no infection should be underestimated. Genetic evaluation of filaggrin mutation can serve as a prognostic tool for diagnosis of atopic diseases. The girl remains under strict medical control and is subjected to integrated therapy of allergic diseases.

The authors declare no conflict of interests.

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