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Features of the SPINK5 gene polymorphism associations with food allergy onset and course in children

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Key words: *Children, Food Allergy, SPINK5-Human Protein, Single Nucleotide Polymorphism.*

Resume. It is less known about role of the genetically predisposing factors in the onset and course of the food allergy (FA).

We **aimed** to determine the association of the polymorphism in *SPINK5 gene* with FA.

Materials and methods. DNA genotypes were identified in children aged 2 months – 5 years of age with FA skin symptoms (n=53) depending on the presence of the guanine or adenine in both alleles in the 1258 position of the 14th exzone.

Results. Genomic frequencies of the *SPINK5* polymorphism site were 15 (28.3%) for AA, 29 (54.7%) – AG, 9 (16.9%) – GG. Abnormal barrier gene STAT5 did not influence on the severity and kind of the FA symptoms, comorbidity. Children with AA<AG genotypes had significantly higher prevalence of food immediate hypersensitivity ($p<0.05$).

Conclusion. Not all food related skin manifestations are AD and small part of them are genetically predisposed.

Асоціація поліморфізму гена SPINK5 із формуванням і перебігом харчової алергії в дітей

О. П. Пахольчук

Роль генетичних факторів у розвитку та формуванні харчової алергії (ХА) залишається маловивченою. Мета роботи – встановити зв'язок поліморфізму гена SPINK5 із ХА. ДНК генотипи залежно від наявності гуаніну та аденіну в алелях у позиції 1258 14-го екзону визначили у 53 дітей у віці від 2 місяців і до 5 років. Генотип АА виявили в 15 (28,3%), АG – 29 (54,7%), GG – 9 (16,9%). SPINK5 генний поліморфізм не впливав на важкість і характер проявів ХА, коморбідність. Діти з АА<АG генотипом частіше мали харчову IgE-асоційовану сенситизацію ($p<0,05$). Встановили, що не всі симптоми, які пов'язані із вживанням їжі, – це АД, лише мала їх частина має генетичну детермінацію.

Ключові слова: *діти, харчова алергія, SPINK5, однонуклеотидний поліморфізм.*

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Ассоциация полиморфизма гена SPINK5 с формированием и течением пищевой аллергии у детей

О. П. Пахольчук

Роль генетических факторов в развитии и течении пищевой аллергии (ПА) остается малоизученной. Цель работы - установить связь полиморфизма гена SPINK5 с ПА. ДНК генотипы в зависимости от наличия гуанина или аденина в аллелях в позиции 1258 14-го экзона определены у 53 детей в возрасте от 2 месяцев до 5 лет. Генотип АА был выявлен у 15 (28,3%), АG – 29 (54,7%), GG – 9 (16,9%). SPINK5 генный полиморфизм не влиял на тяжесть и характер проявлений ПА, коморбидность. Дети с АА<АG генотипом чаще имели пищевую IgE-ассоциированную сенситизацию ($p<0,05$). Установлено, что не все симптомы, связанные с пищей, – это АД, малая их часть генетически детерминирована.

Ключевые слова*: дети, пищевая аллергия, SPINK5, однонуклеотидный полиморфизм.*

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It is well known that skin barrier abnormalities that can be acquired or inherited are linked with severity of the atopic dermatitis (AD). Structural or enzymatic proteins deficiency can lead to the barrier permeability defects. On the other hand AD is only one of the clinical manifestations of the FA on the skin in children. And it is less known about role of the genetically predisposing factors in the onset and course of the food allergy. Although best studied are mutations in filaggrin (FLG), SPINK5 [1].

The skin stratum corneum comprises a multilayered tissue composed of flattened, anucleate corneocytes, surrounded by multiple, planar lamellar sheets, enriched in ceramides, cholesterol, and free fatty acids. It is the localization of these highly hydrophobic lipids within the extracellular domains of the stratum corneum that inhibits water loss. Finally, antimicrobial peptides (AMPs) also are delivered to the stratum corneum intercellular domains through secretion of lamellar body contents [2].

Serine protease inhibitor Karzal type 5 (SPINK5), that is encoded by the gene *SPINK5*, is a protease inhibitor - lymphoepithelial Kazal-type trypsin inhibitor (LEKTI). Several mutations in the *SPINK5* gene are associated with Netherton syndrome. This rare autosomal recessive disorder is characterized by defective cornification of the skin and T_H2-skewed immunologic alterations resembling atopic dermatitis (AD). Lymphoepithelial Kazal-type trypsin inhibitor can influence skin barrier permeability in two different ways. SPINK5 regulates proteolysis in epithelia formation and keratinocyte terminal differentiation, and its defects lead to overdesquamation and skin barrier dysfunction. SPINK5 is also lead to T_H2 responses, such as increased IgE levels and eosinophilia. Elevated serine protease activity likely provokes the barrier abnormality by a second, unrelated mechanism with signaling of the plasminogen activator type 2 receptor (PAR2), which in turn downregulates lamellar body secretion, entombing these organelles in nascent corneocytes. Thus, increased serine protease activity alone induces abnormali-

ties that parallel those in atopic dermatitis [2].

Moreover an epistatic interaction between SPINK5/KLK7 polymorphisms and FLG mutations was detected [3]. Transgenic mice that express human KLK7 display a severe atopic dermatitis-like dermatosis [2]. This lead to the differential diagnosis problems of the allergic skin diseases. And gives opportunity to suppose new diagnostic and therapeutic goals. Association between AD and the G1258A (Glu420Lys) polymorphism in *SPINK5* in a European population was recently reported. Coding polymorphisms in SPINK5 exons 13, 14 and 26 have been reported to be associated with atopic dermatitis (AD), asthma and high level of IgE [4].

However, there have been no reports as to whether the single nucleotide polymorphism (SNP) is associated with the severity of the other allergy skin manifestations and it role in the differential diagnosis of the skin allergy symptoms. AD is one of the different clinical manifestations of the FA and less is known about gene polymorphism association with other FA clinical symptoms.

We therefore **aimed** to determine the association of the polymorphism in *SPINK5 gene* with food allergy onset and features in children from Zaporizhia region. To evaluate it impact in the process of the differential food allergy skin symptoms diagnosis, risk assessment and further perspectives for diagnostics, treatment and prevention.

Materials and methods

Children aged 2 months – 5 years of age with food allergy skin symptoms (n=53) were enrolled in the study. The diagnosis of FA was made basing on the EAACI Guidelines 2014 [5]. It was defined as an episode of immediate allergic reaction, persistent or recurrent, associated with food intake. The diagnosis of AD was made according to the criteria set out by Haniffin and Rajka. After written informed consent was obtained, blood was collected for serum IgE measurement, and DNA extraction. Genomic DNA was extracted from peripheral venous blood using a standard protocol and analized in the Institute of Hereditary Pathology, NAMS of Ukraine, Lviv, Ukraine. Genotyping was performed for SPINK5 – genotype GA (420Glu/Lys), on all 53 individuals. Each child's skin was examined and the clinical symptoms severity were evaluated using SCORAD scale. Transepidermal water lost (TEWL) was evaluated by humidity level, that was measured with skin humidity meter Qeentone, France. Statistical analysis was performed using standard commercial software Statistica (Statsoft, USA). All continuous variables were tested for a normal distribution using the Shapiro-Wilk's W test. The study was approved by the ethics committee of the Zaporizhzhia State Medical University.

Results

After detection of the results the next genotypes were identified depending on the presence of the guanine or adenine in both alleles in the 1258 position of the 14th exzone: AA, AG, GG (*Table 1*).

As it can be seen from the Table 1, genomic frequencies of the *SPINK5* polymorphism site were 15 (28.3%) for AA, 29 (54.7%) for AG, and 9 (16.9%) for GG. As was shown homozygote genotypes were detected rarely than heterozygote one. Distribution of age had no valid differences Abnormal barrier gene SPINK5 did not influence the severity and kind of the FA skin symptoms (*Table 2*).

Table 1

Genotype distribution dependent on sex

	Quantity of the patients(n)	Allele frequency,n(%)		*SPINK5 (*G1258A*) g*enotype frequency					
		A	G	AA		AG		GG	
				n	%	n	%	n	%
Sex M	22	5(22.7%)	17(77.3%)	5	22.7	14	63.7	3	13.6
Sex F	31	10(32.2%)	21(67.8%)	10	32.3	15	48.4	6	19.3
Total	53	15(28.3%)	38(71.7%)	15	28.3	29	54.7	9	16.9

Table 2

***SPINK5 (*G1258A*) g*enotype frequency association with different kind and spread of the FA skin symptoms, comorbid pathology**

	Quantity of the patients (n)	Allele frequency, n/%		*SPINK5 (*G1258A*) g*enotype frequency					
		A	G	AA		AG		GG	
				n	%	n	%	n	%
Skin xerosis	13	4/30.8%	9/69.2%	4	30.8	8	61.5	1	7.7
Palmar dyshidrosis	6	5/83.3%	1/16.7%	1	16.7	4	66.7	1	16.7
Typical AD symptoms	19	7/36.8%	12/63.2%	7	36.8	9	47.4	3	15.8
Local skin symptoms (cheeks, groin region)	17	6/35,3%	11/64,7%	6	35.3	9	52.9	2	11.8
Generalized measles-like eruption	4	4/100%	-	2	50	2	50	-	-
Comorbid pathology AR and/or BOS/BA Dermatophyte infection	21 18	6/28.6% 4/22.2%	1 5/71.4% 14/77.8%	7 4	33.3 22.2	16 11	76.2 61.1	4 3	19 16.7
	21	7/33.3%	14/66.7%	7	33.3	13	61.9	1	4.8
Total	53	15/28.3	38/71.7	15	28.3	29	54.7	9	16.9

Comment: AR-allergic rhinitis, BOS – bronchobstructive syndrome, BA – bronchial asthma.

As it can be seen from the table, SPINK5 gene polymorphism didn't influence on the comorbidity of the FA in patients. This data allow to suppose other kind of interactions of the SNP in studied gene with FA different from that was given for the AD [6]. It is well known that antimicrobial peptides (AMPs) also have relation with this gene function [2]. Prevalence of the Dermatophyte infection had no significant differences depending on SPINK5 genotype.

Except clinical xerosis, transepidermal water lost (TEWL) was evaluated with hydration level. TEWL does not allow the characterization of subsets of patients with or without abnormal gene (p=0.3).

As was given in the literature review SPINK5 gene polymorphism may modulate systemic immune effects favoring the IgE response to atopens [7]. But our results indicated that IgE level and number of ELISA–positive allergens did not differ significantly among genotypes. That corresponds to the other authors' data from the literature review (Kato et al. (2003). It allowed us to suggest that SPINK5 gene polymorphism do not influence on the sensitization process. But deeper analyzes revealed that children with AA<AG genotypes had significantly higher prevalence of food immediate hypersensitivity (p<0.05).

Other authors' studies showed that *SPINK5* doesn't influence on the age of the oral tolerance onset as it was given for STAT6 gene [8]. Most part of those who developed oral tolerance up to 3 y.o. in our research were patients with AG genotype (19/29) and nobody with GG. But age of the children was different so further studies should be provided to support this data.

Discussion

Researches provided in adults in Ukraine showed that GG genotype is associated with risk of atopic dermatitis onset. Healthy people from the control group more frequently had AG genotype (46.9% and 14.3% correspondently) [9]. Our results gives us opportunity to conclude that children with FA more frequently have genotype AG (54.7%), as in healthy population. Associated with AD onset and severity GG genotype was detected only in one fifth of them. Moreover our data showed no associations of the *SPINK5* genotype and food allergy onset, kind of the clinical manifestations and their severity. Taking into account distinguishes in the data concerning SPINK5 SNP influence on the AD features in children and fact that AD is only one of the clinical manifestations of the FA, we can suppose that not all food related skin manifestations are AD and only small part of the patients have inherited skin barrier abnormalities. These facts are very important for further risk assessment. Because as it was previously proven that children with FA have bigger risk of the Bronchial asthma onset, than children with AD (3,1 and 0.5, correspondently) [10].

Further perspectives. Our data correspond to the other author's ones and revealed only association between immediate type of hypersensitivity and AA or AG genotypes. As it was supposed that SPINK5 gene polymorphism may have contribute not only in the skin barrier, but gut permeability too [7]. This kind of the influences should be further studied.

Conclusion

The association of FA wasn't confirmed with SPINK5 polymorphisms. In the 53 children with a food allergy skin symptoms no SNP associations were statistically significant at p<0.05. AA<AG genotype may influence on the gut permeability, it probably have no role in the oral tolerance onset.

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