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ORIGINAL PAPER

Personalized multi-marker panel in the risk assessment of atopic dermatitis phenotypes in children

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ABSTRACT

Introduction: This paper reports the study of a combined genetic and biomarker panel for assessing the risk of development of different phenotypes of atopic dermatitis (AD) in children: alone and combined with other atopic disorders (AtD) – allergic rhinitis/rhino-conjunctivitis (AR/ARC) and bronchial asthma (BA). The aim was to establish a personalized diagnostic multi-marker panel for assessing the developmental risk of different AD phenotypes in children combining single nucleotide polymorphism (SNP) rs_7927894 filaggrin (FLG) genotype variants, serum levels of total immune globulin E (IgE), cutaneous T-cell attracting chemokine (CTACK/CCL27) and thymus and activation regulated chemokine (TARC/CCL17).

Material and methods: The study recruited patients aged 3–18 years old: 39 atopic patients to the main group and 47 non-atopic patients to the control group. All the patients were tested for SNP variants of rs_7927894 FLG and serum concentrations of total IgE, CTACK/CCL27 and TARC/CCL17.

Results: Within AD alone phenotype patients we detected the following significant risk ratios: cytosine\thymine (C/T) rs_7927894 FLG [odds ratio (OR) = 4.14, $p < 0.05$], total IgE > 173 IU/ml (OR = 8.98, $p < 0.001$), CTACK/CCL27 > 3658.5 pg/ml (OR = 5.64, $p < 0.01$). Atopic disorders combined with other AtD phenotype: C/T rs_7927894 FLG (OR = 2.88, $p < 0.05$), total IgE > 213 IU/ml (OR = 136.7, $p < 0.001$), CTACK/CCL27 > 4308.8 pg/ml (OR = 7.40, $p < 0.001$). With AD combined with other AtD collated to AD alone – total IgE > 1001 IU/ml (OR = 16.0, $p < 0.001$). TARC/CCL17 had no significant differences among main and control groups.

Conclusions: Cytosine\thymine rs_7927894 FLG variant combined with cut-off serum IgE and CTACK/CCL27 levels is a novel significant personalized multi-marker panel for assessing the risk of development of the different AD phenotypes in children.

KEY WORDS:

filaggrin, total IgE, atopic dermatitis, CTACK/CCL27, TARC/CCL17.

INTRODUCTION

Atopic dermatitis (AD) is a chronic relapsing skin disease with underlying mechanisms of allergic inflammation, globally affecting over 20% of the pediatric population [1]. The natural progression of AD to allergic rhinitis/rhino-conjunctivitis (AR/ARC) and then, finally, to bronchial asthma (BA) is called the "atopic march". However, a study aiming to more profoundly understand the mechanisms of atopy progression analyzed the data from the Avon Longitudinal Study of Parents and Children and Manchester Asthma and Allergy Study and revealed eight separate profiles of the comorbid combinations of AD, AR and wheezing [2]. Regarding the above,

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there is a novel approach applied to AD and its atopic comorbidities, particularly BA: the terminology "AD phenotypes" [2, 3]. Atopic patients could be divided into several phenotypes: AD only, AD with AR/ARC, AD with AR/ARC and BA, AD with BA.

From our own clinical practice and foreign literature data [4] there emerges the understanding of a decreased plausibility of total immune globulin E (IgE) as the basic and specific serum biomarker (SBM) to evidence the severity of allergic inflammation in the skin and airways within different AD phenotypes. Thus, patients with moderate-to-severe signs of AD and high scores for the atopic dermatitis (SCORAD) index have a total serum IgE level that is normal or slightly exceeding the normal values and, vice versa, there is an elevation of total serum IgE within physiologic or trending to atopy skin and zero SCORAD. One of the novel studies points out the significant correlation between the development of contact AD to house dust mites and SCORAD level both within children having elevated total IgE (along with the specific IgE to the aforesaid allergens) and children having the bespoke biomarkers not elevated [5].

The above considerations necessitate the need of a novel SBM for assessing the severity degree of AD within its phenotypes alone or combined with AR/ARC and/or BA. In this regard there are chemokines (CC) that have been actively studied during the last few decades – cutaneous T-cell attracting chemokine or C-C motif chemokine ligand 27(CTACK/CCL27) and thymus and activation regu¬lated chemokine or C-C motif chemokine ligand 17 (TARC/CCL17). The serum levels of the bespoke CC are associated with intensity of the AD clinical manifestations [6].

CTACK/CCL27 attracts T-cells expressing the cutaneous lymphocyte antigen which is involved in the AD pathogenesis. It is a 112 amino acid long peptide whose gene resides in the 9pq13 chromosomal region, the CC cluster [7]. As a ligand it is selectively produced in the skin, specifically increasing within AD, enhancing the T-helper-2 (Th_2) mediated inflammation [8]. Machura *et al*. (2012) detected that CTACK/CCL27 along with TARC/CCL17 was elevated in sera of patients suffering from pediatric AD compared to pediatric BA, urticaria patients as well as healthy controls [9]. Regarding the above, CTACK/CCL27 is revealed as playing one of the pivotal roles in AD pathogenesis.

TARC/CCL17 is produced by keratinocytes, dendritic cells, endothelial cells and fibroblasts, being activated in the thymus [7]. It is encoded by the respective gene residing on chromosome 16q13, and it serves as a chemoattractor for Th_2 cells [4, 7]. TARC/CCL17 receptors are located on CCR4-positive Th_2 cells. Murine models showed that TARC/CCL17's expression is elevated within AD by basal keratinocytes as well as by the endothelial cells of the dermis vessels [4]. TARC/CCL17 injection induces migration of Th_2 cells and production of interleukin-4 and is associated with the severity of AD [7]. Thus, TARC/CCL17 is a proven SBM of AD activity and inflammation severity [4, 7, 9, 10]. A meta-analysis appraising 3 electronic databases comprising 222 articles, concerning 115 different biomarkers studied in 30 063 patients with AD proved the pivotal role of TARC/CCL17 in AD clinical outcomes with the highest correlation ratios; CTACK/CCL27 was detected as useful, though still requiring further studies [11].

Besides the underlying serum mechanisms AD possesses a profound and complex genetic background. The filaggrin (*FLG*) gene residing in chromosome region 1q23.1 and its single nucleotide polymorphisms (SNP) were detected as the most robustly associated with AD manifestations [11]. The most common atopic phenotypes associated with the bespoke SNP are AD with BA, rather than AD alone [12, 13].

Filaggrin, emerging from an inactive precursor pro-FLG molecule by the cascade of proteolytic and dephosphorylating reactions, is the main component of the natural moisturizing factor (along with hyaluronic acid, lactate, sodium, potassium, magnesium, phosphate, calcium, and citrate) which binds to the intermediate filaments the keratin-producing micro-fibrils, transforming keratinocytes into flattened corneocytes. Thus, the trans-epidermal water loss is restored and the normal functioning of the skin stratum corneum is achieved [14]. Single nucleotide polymorphism of *FLG* are the most studied culprit for AD development, particularly *FLG* rs_7927894 [15, 16]. One of the latest studies conducted by Dębińska *et al*. (2020), based on the preceding genome-wide studies, detected the *FLG* rs_7927894 T-allele as mostly associated with pediatric AD [16].

As yet there are scarce data on combining the genetic markers and SBM into a multi-marker panel which would make it possible to predict the developmental risk of AD in its different phenotypes with a higher level of precision and personalization. Nevertheless, a panel comprising *FLG* rs_7927894 allele variants (C/C – cytosine/cytosine, C/T – cytosine\thymine, T/T – thymine/thymine), total IgE, CTACK/CCL27 and TARC/CCL17 serum concentrations is emerging as a perspective tool for studying pediatric AD cohorts.

Consequently, the aim of the current study was to establish a novel multi-marker panel assessing the developmental risk of different AD phenotypes by detecting the carriage of SNP rs_7927894 *FLG* genotype variants combined with serum concentrations of IgE total, CTACK/CCL27 and TARC/CCL17 in children aged 3–18 years.

MATERIAL AND METHODS

We enrolled 86 pediatric patients in the study: 39 atopic children in the main group and 47 non-atopic children in the control group.

The main group (n = 39) was composed of patients suffering from AD of different severity degrees (mild,

moderate and severe), which had been assessed clinically using the SCORAD scale (< 20 points – mild degree, 20–40 points – moderate degree, > 40 points – severe degree) [17]. Inclusion criteria were as follows: age 3–18 years, the established diagnosis of AD alone or with AR/ARC and/or BA. Exclusion criteria were as follows: age below 3 or above 18 years, no AD manifestations (0 points on SCORAD scale). Those patients were enrolled at the Department of Pediatrics 1 and Medical Genetics of Dnipro State Medical University (Dnipro, Ukraine), as well as in-patient and out-patient departments of the Allergy Centre of MNPE "Clinical Hospital of the Emergency Medicine" of Dnipro City Council.

The control group (n = 47) was composed of patients suffering from digestive system disorders (DSD): functional dyspepsia, gastro-esophageal reflux disease, gastritis, functional disorders of the biliary system. Inclusion criteria were as follows: age 3–18 years, a confirmed diagnosis of DSD, no atopic disorder (AtD) manifestations (AD, AR/ARC, BA). Exclusion criteria were as follows: age below 3 or above 18 years, manifesting AtD (AD, AR/ARC, BA). Those patients were enrolled at the Pediatric Gastroenterology Department of the MNPE "City Clinical Hospital #9" of Dnipro City Council.

Patients of the main and control groups underwent buccal swabs, which were then frozen and kept within the temperature range from –18°C to –32°C. Then, they were transported within temperature maintenance to the Department of General and Molecular Pathophysiology of Bohomoletz Institute of Physiology (National Academy of Sciences, Kyiv, Ukraine) to undergo the real-time polymerase chain reaction.

Determination of *FLG* rs_7927894 SNP variants was carried out using the TaqMan allelic discrimination assay C_3243267_10 with the Applied Biosystems 7500 Fast Real Time PCR System [18].

Patients of the main and control groups gave venous blood samples which were then tested for serum levels of total IgE, CTACK/CCL27 and TARC/CCL17. The samples were transported, frozen and processed in the certified laboratory using the IgE Elecsys 2010 Immunoassay (serial No. 04827031), RayBio Human CTACK/CCL27 ELISA Kit (ELH-CTACK/CCL27, ISO 13485 Certified, Lot 013018 0236) and RayBio Human TARC/CCL17 ELISA Kit (ELH-TARC/CCL17, ISO 13485 Certified, Lot 013018 0311).

The statistical significance of the results was assessed using specific statistic tools (StatSoft Inc., USA, license # AGAR909E415822FA). The normality of quantitative data for the population distribution within the patient cohorts was estimated by applying the Kolmogorov-Smirnov test (K-S test, one-sample), Shapiro-Wilk test and Lilliefors test. The statistical significance of the categorical variables between the main and control group cohorts was verified by Pearson's χ^2 test within cell frequencies exceeding 5 and otherwise the two-tailed Fisher's exact test as well as by Student's t-test for comparison of mean values. For establishing the cut-off figures, diagnostic specificity and sensitivity of the studied SBM (IgE total, CTACK/CCL27, TARC/CCL17) we applied the receiver operator characteristics (ROC) plot, evaluating the area under the curve square with 95% confidence interval (95% CI). To calculate the association of serum IgE total, CTACK/CCL27 and TARC/CCL17 with different AD phenotypes' development risk we applied the odds ratio (OR) value with the 95% CI. In validating all the data obtained the significance level was set at 0.05 ($p < 0.05$).

The patients' legal representatives duly filled in and signed the informed consent forms according to the Declaration of Helsinki (last revised – 64th WMA General Assembly, Fortaleza, Brazil, October 2013), and the study design and methods were approved by the Local Ethics Committee of Dnipro State Medical University (Minutes #7 as of October 28th, year 2020).

DISCLOSURE STATEMENT

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RESULTS

The gender distribution did not reveal any significant differences between the main and control group patients (Table 1).

Hence, the data surveilled in the first part of Table 1 depict a slight prevalence (< 10%) of male patients over female ones in the main group with AD and vice versa – in the control group of non-atopic patients.

When analyzing the age distribution within the total overall and control groups, we detected the significantly highest frequency of the age cohort as 7–11 years old among atopic patients and of 12–18 years old among the non-atopic patients. The incidence of AD in its different phenotypes was also significantly higher in the age cohorts of 4–6 years and 7–11 years old.

The mean age of AD patients was lower compared to the DSD patients – 7.8 (95% CI: 6.7–8.9) and 10.9 (95% CI: 9.7–12.1) years old respectively (Student's t-test, $p < 0.001$). This confirms the hypothesis that AD starts earlier within the age collated to DSD.

We detected a significant positive correlation of C/T rs_7927894 *FLG* genotype and all the studied AD phenotypes ($r = 0.29$, $p < 0.05$). Also, the aforesaid genotype

TABLE 1. Gender and age distribution among patients of the main and control groups

Parameters	Main group		Control group	
	n	%	*n*	%
Gender				
Male*	21	53.85	21	44.68
Female*	18	46.15	26	55.32
Total	39	100.00	47	100.00
AGE				
0–3 years old**	2	5.13	1	2.13
4–6 years old***	13	33.33	8	17.02
7–11 years old***	19	48.72	16	34.04
12–18 years old***	5	12.82	22	46.81
Total	39	100.00	47	100.00

** Validated by Pearson's χ^2 test, $p > 0.05$*
*** Validated by Fisher's exact test, two-tailed, $p < 0.05$*
**** Validated by Pearson's $\chi 2$ test, $p < 0.01$*

variant was detected to be the significantly most frequent genotype among AD patients (Figure 1). Other data point out the trending to significance negative correlation of C/C rs_7927894 *FLG* genotype with AD incidence ($r = -0.19$, $p = 0.07$). The C/C variant was detected as the most frequent SNP rs_7927894 *FLG* within the DSD patients (Figure 1). Thymine/thymine rs_7927894 *FLG* genotype did not show any significant correlations either with AD or with DSD patients ($p > 0.05$) and was detected most seldom of all the studied variants.

Table 2 provides the mean SBM figures for the patients of the overall main and control groups.

Serum level of IgE total was significantly 21.9-fold higher in the main group in comparison with control group DSD patients, which reveals atopic inflammation within AD patients. Serum level of CTACK/CCL27 was significantly detected more than 1.26-fold higher in the main group than in the control one, which supports the specificity of CCL27 as the SBM for AD in children. The data on TARC/CCL17 serum concentrations did not reveal any statistically significant differences in the CCL17 levels between the main and control group patients, and the difference between the main and control groups' values did not exceed 0.05-fold.

The applied ROC analysis yielded the SBM cut-off values serving as tools for the AD phenotypes' development risk assessment in the main group cohorts compared to the control group: AD alone (18 patients), AD + AtD (21 patients) and DSD (47 patients), as shown in Table 3. Thus, significant SBM cut-off values for AD alone phenotype are: > 173 IU/ml for IgE total and > 3658.5 pg/ml for CTACK/CCL27. The significant cut-off values for AD + AtD phenotype patients are: > 213 IU/ml for IgE total and > 4308.8 pg/ml for CTACK/CCL27. Finally, collation of AD alone and AD + AtD phenotype cohorts (AR/ARC and/or BA) yielded the significant cut-off > 1001 IU/ml for total

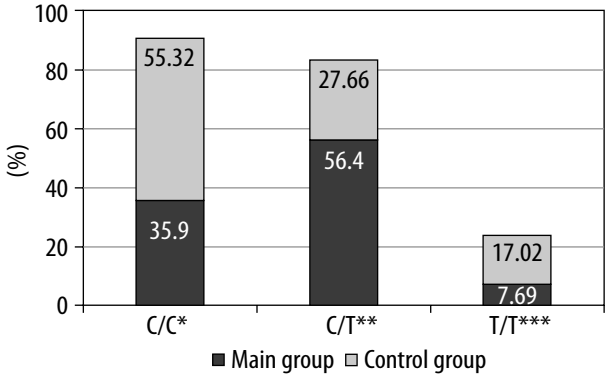

FIGURE 1. Single nucleotide polymorphism rs_7927894 filaggrin genotype variants' frequency in the overall main and control groups

C/C – cytosine/cytosine, C/T – cytosine\thymine, T/T – thymine/thymine
** Validated by Pearson's χ^2 test, $p = 0.07$*
*** Validated by Pearson's χ^2 test, $p < 0.01$*
**** Validated by Pearson's χ^2 test, $p > 0.05$*

TABLE 2. Serum biomarkers in patients of the main and control groups

Groups/values	IgE total [IU/ml], median (LQ; HQ)	CTACK/CCL27 [pg/ml], median (LQ; HQ)	TARC/CCL17 [pg/ml], mean (95% CI)
Main group	679.6 (212.2; 1363.0)	4403.6 (3726.2; 5148.7)	615.8 (523.4–708.2)
Control group	31.1 (14.0; 130.2)	3495.9 (3197.8; 4186.8)	608.4 (543.8–673.0)
Statistical significance	< 0.001*		> 0.05**

CTACK/CCL27 – cutaneous T-cell attracting chemokine, HQ – higher quartile, IgE – immune globulin E, LQ – lower quartile, TARC/CCL17 – thymus and activation regulated chemokine
** Validated by Mann-Whitney U test*
*** Validated by Student's t-test*

TABLE 3. Cut-off values, specificity and sensitivity of serum biomarkers for atopic dermatitis phenotypes in children

Value/cut-off value	Area under the curve, *n* (95% CI)	Statistical significance (area = 0.5)	Specificity (95% CI)	Sensitivity (95 % CI)
Main group AD alone vs. control group				
IgE TOTAL > 173 IU/ml	0.748 (0.625–0.848)	< 0.001	85.1 (71.7–93.8)	61.1 (35.8–82.6)
CTACK/CCL27 ≥ 3658.5 pg/ml	0.720 (0.595–0.824)	< 0.01	61.7 (46.4–75.5)	77.8 (52.4–93.5)
Main group AD + AtD vs. control group				
IgE TOTAL > 213 IU/ml	0.948 (0.866–0.987)	< 0.001	87.2 (74.2–95.1)	95.2 (76.1–99.2)
CTACK/CCL27 ≥ 4308.8 pg/ml	0.748 (0.628–0.846)	< 0.001	78.7 (64.3–89.3)	66.7 (43.0–85.4)
Main group AD alone vs. main group AD + AtD				
IgE TOTAL > 1001 IU/ml	0.799 (0.640–0.910)	< 0.001	88.9 (65.2–98.3)	66.7 (43.0–85.4)

AD – atopic dermatitis, AtD – atopic disorders, CTACK/CCL27 – cutaneous T-cell attracting chemokine, IgE – immune globulin E

TABLE 4. The developmental risk of atopic dermatitis phenotypes in children based on genotype and serum biomarkers' combination

Cohorts/values	C/T rs_7927894 FLG		IgE TOTAL > 173 IU/ml		CTACK/CCL27 ≥ 3658.5 pg/ml	
	Yes	No	Yes	No	Yes	No
Main group: AD, *n* = 18 (%)	61.1	38.9	61.1	38.9	77.8	22.2
Control group, *n* = 47 (%)	27.7	72.3	14.9	85.1	38.3	61.7
OR (95 % CI)	4.11 (1.28–13.18)		8.98 (2.53–31.86)		5.64 (1.56–20.32)	
Statistical significance	χ^2, $p < 0.05$		χ^2, $p < 0.001$		FET, $p < 0.01$	
	C/T rs_7927894 FLG		IgE TOTAL > 213 IU/ml		CTACK/CCL27 ≥ 4308.8 pg/ml	
	Yes	No	Yes	No	Yes	No
Main group: AD + AtD, *n* = 21 (%)	52.4	47.6	95.2	4.8	66.7	33.3
Control group, *n* = 47 (%)	27.7	72.3	12.8	87.2	21.3	78.7
OR (95 % CI)	2.88 (1.07–8.54)		136.67 (14.77–1264.24)		7.40 (2.30–23.76)	
Statistical significance	χ^2, $p < 0.05$		FET, $p < 0.001$		χ^2, $p < 0.001$	
	IgE TOTAL > 1001 IU/ml					
	Yes		No			
Main group: AD , *n* = 18 (%)	11.1		88.9			
Main group: AD + AtD, *n* = 21 (%)	66.7		33.3			
OR (95 % CI)	16.0 (2.68–95.44)					
Statistical significance	FET, $p < 0.001$					

AD – atopic dermatitis, AtD – atopic disorders, C/T – cytosine\thymine, a pathologic single nucleotide variation (SNV), CTACK/CCL27 – cutaneous T-cell attracting chemokine, FET – Fisher's exact test, IgE – immune globulin E, OR – odds ratio

IgE as the SBM predicting the developmental risk of AD combined with AtD rather than AD alone phenotype.

Finally, we applied regression analysis to the studied patient's values of the main and control groups to quantify the OR for developmental risk of different AD phenotypes (Table 4).

In atopic dermatitis alone phenotype cohort analysis, the genotype variant C/T rs_7927894 *FLG* significantly prevailed among AD alone patients compared to the controls and showed a significant 4.11-fold higher developmental risk of the studied phenotype. Total IgE > 173 IU/ml significantly prevailed among the AD alone collated with control group patients and showed a 8.98-fold higher developmental risk for the bespoke AD phenotype. CTACK/CCL27 > 3658.5 pg/ml was also significantly more frequent among AD alone patients compared to controls and showed a 5.64-fold higher developmental risk for developing the AD alone phenotype.

In AD + AtD phenotype cohort analysis, the genotype variant C/T rs_7927894 *FLG* yielded a higher incidence within AD + AtD patients over non-atopic controls along with the total IgE > 213 IU/ml, which revealed significantly higher prevalence among the atopic patients than controls, having significant, extremely high developmental risk, 136.7-fold higher. CTACK/CCL27 > 4308.8 pg/ml was significantly much more frequent within AD + AtD patients compared to the control group, with significant 7.40-fold increased risk for developing the bespoke phenotype.

Finally, the collation of the AD alone to AD + AtD patient cohorts of the main group by the single cut-off

value – total IgE > 1001 IU/ml – yielded its significantly higher incidence in the AD + AtD patients compared to AD alone patients along with the significant 16.0-fold higher developmental risk for the aforesaid phenotype.

DISCUSSION

The gender distribution data as shown in Table 1 support the hypothesis that male gender is more vulnerable to AtD than female. Still, considering the statistical non-significance, it necessitates confirmation by further studies on larger patient cohorts.

The recent studies focusing on the genetic basis of AD are evidencing the pivotal role of SNP rs_7927894 *FLG* in the genetic mechanisms of AD Particularly, Dębińska *et al*. (2020) detected higher frequency of the T-allele rs_7927894 *FLG* within atopic compared to non-atopic patients. This partly agrees with the present study to have detected diploid heterozygote variant C/T rs_7927894 *FLG* as the most frequent and culprit one in the developmental risk of pediatric AD phenotypes [15, 16].

In our study we detected that serum total IgE and CTACK/CCL27 concentrations in patients suffering AD alone or in combination with other AtD were significantly higher than in non-atopic patients of the control group. Machura *et al*. (2012) observed elevated serum TARC/CCL17 and CTACK/CCL27 concentrations in children with AD, allergic BA and urticaria separately [9]. However, in the present study we did not detect any significant difference in the serum TARC/CCL17 concentration between AD combined with BA patients and the controls without atopy. The cause of this data discrepancy is that the present study recruited patients suffering from atopic BA combined with AD as the phenotype-determining disorder into the main group and DSD patients as controls, whereas Machura *et al*. [9] recruited patients suffering AD, BA and urticaria as separate phenotypes along with the healthy children as controls. This points to the need for further studies involving similarly stratified atopic patients of separate AD, AR/ARC and BA phenotypes to detect the serum total IgE, CTACK/CCL27 and TARC/CCL17 cut-off values along with OR developmental risks of the mentioned atopic phenotypes. This will allow us to exclude data discrepancies and validate the results of our study with ones similar in design to Machura *et al*. [9].

Kataoka's research of TARC/CCL17 levels within AD (2014) detected it as the most sensitive SBM for the disease [4]. It indicates that monitoring serum TARC/CCL17 level is an effective tool to evaluate initial AD activity and its treatment efficacy, prevent further food allergy progression and improve AD prognosis within infants. Still, in our present study the mean figures of TARC/CCL17 reached 615.8 (95% CI: 523.4–708.2) pg/ml in AD patients and 608.4 (95% CI: 543.8–673.0) pg/ml in the DSD controls. This deviates from Kataoka's study results of normal serum TARC/CCL17 figures < 743 pg/ml in children aged 2–15 years old and could be due to the different TARC/CCL17 production rates in the healthy children and AD patients within different ethnic groups and necessitates multi-center comparative studies recruiting multi-racial cohorts.

Still, none of the aforementioned studies investigated the multi-marker panel of genotype C/T rs_7927894 *FLG* combined with SBM total IgE, CTACK/CCL27 to provide a systemically backgrounded, more personalized prognosis for the development risk of AD phenotypes as has been carried out in our study. This points to the significant validity and the possibility to launch a combined panel consisting of C/T rs_7927894 *FLG* genotype, serum total IgE and CTACK/CCL27 as a novel developmental risk assessment multi-marker panel for different AD phenotypes in children.

CONCLUSIONS

The novel significant personalized multi-marker panel for assessing the developmental risk of AD alone and AD combined with other AtD phenotypes in children comprises the C/T rs_7927894 *FLG* variant combined with elevated serum total IgE and CTACK/CCL27 levels.

To detect the significant risk of developing the AD alone phenotype in children a pediatric allergologist should determine the following multi-marker panel: C/T rs_7927894 *FLG* genotype variant, serum total IgE > 173 IU/ml along with CTACK/CCL27 > 3658.5 pg/ml.

To detect the significant risk of developing the AD combined with other AtD (AR/ARC and/or BA) phenotype in children a pediatric allergologist should determine the following multi-marker panel: C/T rs_7927894 *FLG* genotype variant, serum total IgE > 213 IU/ml and CTACK/CCL27 > 4308.8 pg/ml.

To confirm the significant prevalence of developmental risk for AD combined with other AtD phenotype (AR/ARC and/or BA) collated to AD alone phenotype in children a pediatric allergologist should additionally determine serum total IgE > 1001 IU/ml in the blood analysis.

DISCLOSURE

The authors declare no conflict of interest.

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