Genotype Associations with the Different Phenotypes of Atopic Dermatitis in Children

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ABSTRACT

This study deals with detecting the associations of atopic dermatitis' (AD) phenotypes in children: alone or combined with seasonal allergic rhino-conjunctivitis (SARC) and/or perennial allergic rhinitis (PAR), and/or with bronchial asthma (BA) with single nucleotide polymorphisms (SNP) of filaggrin (*FLG*), thymic stromal lymphopoietin (*TSLP*) and orsomucoid-like-1 protein 3 (*ORMDL3*) genes.

Male and female pediatric patients aged from 3 to 18 years old were recruited into the main (AD in different combinations with SARC, PAR, BA) and control groups (disorders of digestives system, neither clinical nor laboratory signs of atopy). Patients were genotyped for SNP of rs_7927894 *FLG*, rs_11466749 *TSLP*, rs_7216389 *ORMDL3* variants.

Statistically significant associations of the increased risk were detected of AD combined with SARC and/or PAR and AD combined with BA (possibly, SARC and/or PAR) with C/T rs_7927894 *FLG*and T/T rs_7216389 *ORMDL3* genotypes. Genotype C/C rs_7927894 *FLG* significantly decreases the risk of AD combined with SARC and/or PAR by 2.56 fold.

Several genotypes' associations had a trend to significance: C/C rs_7216389 *ORMDL3* decreases and C/T rs_7216389 *ORMDL3* increases the risk for developing AD alone phenotype; A/G rs_11466749 *TSLP* decreases the risk of AD combined with BA (possibly, SARC and/or PAR) phenotype development.

KEYWORDS

atopic dermatitis; children; genotype; phenotype; associations; filaggrin; thymic stromal lymphopoietin; orsomucoid1-like protein 3

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Received: 31 January 2020
Accepted: 22 February 2021
Published online: 30 July 2021

Acta Medica (Hradec Králové) 2021; 64(2): 96–100
https://doi.org/10.14712/18059694.2021.17

INTRODUCTION

Atopic dermatitis is a common, chronic, relapsing, inflammatory skin disease that primarily affects young children with an underlying inherited tendency to produce immunoglobulin E (IgE) antibodies in response to minute amounts of common environmental proteins (1), basically of food origin. AD clinically manifests with skin itching, skin lesions, dryness or oozing and rash of a multiple morphology and localization. It can induce the development of other atopic disorders (AtD) in children: seasonal allergic rhino-conjunctivitis (SARC), perennial allergic rhinitis (PAR) and bronchial asthma (BA). Such the phenomenon of AD progression into other AtD is defined as atopic march (AM) (2). Within different models of atopy progression there is an evident linkage between AD and AtD in the upper and lower airways (2, 3). Still, some studies directly point out at the oversimplification of AM hypothesis as an approach for interpreting the progression of AtD in children – there is being introduced the phenomenon of personalized AD phenotypes in combination with SARC and/or PAR, and/or BA (4, 5).

It was demonstrated that pathogenesis of AD and other AtD is preceded by a genetic predisposition and the set of major candidate-genes involved is being updated at the current moment (6). The recent studies yielded an evidence that genes to have such associations are: filaggrin (*FLG*) (2, 7, 8), thymic stromal lymphopoietine (*TSLP*) (8–10), orsomucoid1-like protein 3 or sphingolipid biosynthesis regulator 3 (*ORMDL3*) (11). Despite that *FLG* mutations are being studied longitudinally with much data collected so far (2, 6, 8), still there is a lack of studies on detection of the role of particular single nucleotide polymorphisms (SNP) of *FLG*, *TSLP* and *ORMDL3* genes in pathogenesis of AD and its phenotypes at pediatric patients. Accordingly, the associations and their character – increasing or decreasing – of the mentioned genes SNP with the risk of developing respective AD phenotypes need a novel elucidation.

Given the aforesaid, study goal was to detect the associations of different AD phenotypes with SNP rs_7927894 *FLG*, rs_11466749 *TSLP*, rs_7216389 *ORMDL3* in children.

MATERIALS AND METHODS

We had recruited 95 patients into the main group suffering the AD alone (n = 47) and in different combinations: a cohort of AD combined with either SARC and/or PAR (n = 38), AD combined with BA (possibly, with SARC and/or PAR) (n = 10). The patients were aged from 3 to 18 years old, age median had been 8 years old (LQ–HQ: 5–11). They were being recruited at Department of pediatrics 1 and medical genetics of SE "Dnipro medical academy of Health Ministry of Ukraine", in-patient and out-patient departments of the Allergy Centre of MNCE "Clinical hospital of the emergency care" of Dnipro City Council". The inclusion criteria consisted of: age 3–18 years old, the officially established diagnosis of AD, AD with SARC, AD with PAR, AD with SARC and/or PAR, AD with BA (possibly, SARC and/or PAR), elevated serum total IgE (>100 IU/ml). The exclusion criteria comprised absence of skin or airways' AtD's specific clinical signs, not elevated serum total IgE (<100 IU/ml).

The control group consisted of 80 patients not suffering from AtD recruited at the Department of pediatric gastroenterology of the MNCE "City clinical hospital #1" of Dnipro City Council". The children were aged from 3 to 18 years old and had been suffering the following diseases of gastro-intestinal tract: functional dyspepsia, chronic gastritis, peptic ulcer, gastro-esophageal reflux disease, functional disorders of the biliary system. The inclusion criteria were as follows: no clinical signs of AD, SARC, PAR or BA at the moment of enrollment into the study as well as in case history, not elevated IgE (<100 IU/ml).

Patients of all the groups had undergone the buccal swab, the material then had been consequently stored within a temperature range from –18° to –32° centigrade in the freezer; afterwards the material had been studied by genotyping using the method of allele discrimination analysis based on polymerase chain reaction in real time (qPCR). The genotyping was carried out on the Applied Biosystems 7500 Fast Real Time PCR System (12) using rs_7927894, rs_11466749, rs_7216389 TaqMan® allelic discrimination assays: C__3243267_10, C__29062108_10 and C__31152869_10 respectively.

All the patients had the informed consent duly filled in (signed by their parents or legal representatives). All the study was performed according to the Declaration of Helsinki (52nd WMA General Assembly, Edinburgh, Scotland, October 2000), and the procedures have been approved by the local ethics committee of the SI "Dnipropetrovsk medical academy of HM of Ukraine".

To verify the difference of relative values statistical significance, we had applied the Pearson's chi-squared test ($\chi 2$) and Fischer' s exact test, two-tailed (FET, for small values, $n < 5$), verified by the p-value level ($p < 0.05$). For detection of associative relationship between the values of the main and control groups we applied the Pearson' s contingency coefficient (rc). To calculate relative risks of AD phenotypes' association with SNP we applied the Odds ratio (OR) value with the respective 95% confidence interval (95% CI), the significance having been evidenced by p-value level ($p < 0.1$).

RESULTS

There was received the following age-gender distribution of the patients in the main and control groups (Tables 1, 2).

As it can be distinctly seen from Table 1, we detected the prevalence of male atopic patients compared to females in the most of cohorts of the main group, excluding the AD combined with BA (possibly, SARC and/or PAR) cohort.

In the age distribution the most frequent age interval was 7–11 years old in all the main group cohorts, therefore in the control group there had been detected the prevalence of patients aged 12–18 years old.

Tab. 1 The gender distribution among patients of the main and control groups.

	MAIN GROUP, COHORTS				
GENDER	AD	AD +SARC / AD + PAR / AD + SARC +PAR	AD + BA (SARC and/or PAR)	AD PHENOTYPES TOTAL	CONTROL GROUP
Patients total (N)	47	38	10	95	80
MALE, N	27	25	4	56	47
MALE, %	57.4%	65.8%	40.0%	58.9%	58.8%
FEMALE, N	20	13	6	39	33
FEMALE, %	42.6%	34.2%	60.0%	41.1%	41.2%

Tab. 2 The age distribution in patients of the main and control groups.

AGE, YEARS OLD, N/%	MAIN GROUP, COHORTS				
	AD	AD +SARC / AD + PAR / AD + SARC +PAR	AD + BA (SARC and/or PAR)	AD PHENOTYPES TOTAL	CONTROL GROUP
0–3, N	6	1	0	7	2
0–3, %	12.8%	2.6%	0.0%	7.4%	2.5%
4–6, N	18	8	0	26	13
4–6, %	38.3%	21.1%	0.0%	27.4%	16.2%
7–11, N	16	17	6	39	23
7–11, %	34.0%	44.7%	60.0%	41.0%	28.8%
12–18, N	7	12	4	23	42
12–18, %	14.9%	31.6%	40.0%	24.2%	52.5%
Me, years (LQ–HQ),	6 (4–10)	9 (7–12)	11 (10–12)	8 (5–11)	12 (9–15)

ASSOCIATIONS OF AD ALONE WITH SNP rs_7927894 *FLG*, rs_11466749 *TSLP* AND rs_7216389 *ORMDL3*

The data in Table 3 show no statistically significant association of the AD alone phenotype development risk with any of the genes-candidiates. Still, trends to statistical significance with this phenotype were detected (p-value between 0.05 and 0.1): with C/T rs_7216389 *ORMDL3* genotype – the maximal risk (OR = 2.14 (95% CI 0.98, 4.65)), and with C/C rs_7216389 *ORMDL3* genotype – minimal risk (OR = 0.41 (95% CI 0.16, 1.04)).

Tab. 3 Associations and frequency of SNP rs_7927894 *FLG*, rs_11466749 *TSLP* and rs_7216389 *ORMDL3* with AD alone phenotype in children.

Cohorts	Genotypes SNP rs_7927894 *FLG*		
	C/C	C/T	T/T
Main group	36.2%	46.8%	17.0%
Control group	47.5%	32.5%	20.0%
Statistical significance by Pearson χ2-test	p > 0.05	p > 0.05	p > 0.05
	Genotypes SNP rs_11466749 *TSLP*		
	A/A	A/G	G/G
Main group	55.3%	42.6%	2.1%
Control group	56.3%	40.0%	3.7%
Statistical significance by Pearson χ2-test (* FET, two-tailed)	p > 0.05	p > 0.05	p > 0.05*
	Genotypes SNP rs_7216389 *ORMDL3*		
	C/C	C/T	T/T
Main group	14.9%	72.3%	12.8%
Control group	30.0%	55.0%	15.0%
Statistical significance by Pearson χ2-test	p = 0.0557	p = 0.0526	p > 0.05
OR (95% CI)	0.41 (0.16; 1.04)	2.14 (0.98; 4.65)	
PCC (rc)	–0.170	0.172	

ASSOCIATIONS OF AD COMBINED WITH SARC AND/OR PAR PHENOTYPE WITH SNP rs_7927894 *FLG*, rs_11466749 *TSLP* AND rs_7216389 *ORMDL3*

Data obtained shows that patients carrying C/C rs_7927894 *FLG* genotype have significantly decreased risk by 2.56 fold of AD onset (OR = 0.39 (95% CI 0.17, 0.92); rc = –0.202; p < 0.05); patients carrying C/T rs_7927894 *FLG* genotype have the significantly increased risk by 2.57 fold (OR = 2.57 (95% CI 1.1, 5.67); rc = 0.217; p < 0.05) and carriers of T/T rs_7216389 *ORMDL3* genotype – increased risk by 3.31 fold (OR = 3.31 (95% CI 1,34; 8,14); rc = 0,246; p < 0.01) for developing the AD combined with SARC and/or PAR phenotype.

Tab. 4 Associations and frequency of SNP rs_7927894 FLG, rs_11466749 *TSLP* and rs_7216389 *ORMDL3* with AD combined with SARC and/or PAR phenotype in children.

Cohorts	Genotypes SNP rs_7927894 *FLG*		
	C/C	C/T	T/T
Main group	26.3%	55.3%	18.4%
Control group	47.5%	32.5%	20.0%
Statistical significance by Pearson χ2-test	p < 0.05	p < 0.05	p > 0.05
OR (95% CI)	0.39 (0.17; 0.92)	2.57 (1.16; 5.67)	0.39
PCC (rc)	–0.202	0.217	
	Genotypes SNP rs_11466749 *TSLP*		
	A/A	A/G	G/G
Main group	60.5%	31.6%	7.9%
Control group	56.3%	40.0%	3.7%

Cohorts	Genotypes SNP rs_11466749 *TSLP*		
	A/A	A/G	G/G
Statistical significance by Pearson χ2-test (* FET, two-tailed)	p > 0.05	p > 0.05	p > 0.05*
	Genotypes SNP rs_7216389 *ORMDL3*		
	C/C	C/T	T/T
Main group	18.4%	44.7%	36.9%
Control group	30.0%	55.0%	15.0%
Statistical significance by Pearson χ2-test	p > 0.05	p > 0.05	p < 0.01
OR (95% CI)			3.31 (1.34;8.14)
PCC (rc)			0.246

ASSOCIATIONS OF AD COMBINED WITH BA (POSSIBLY, SARC AND/OR PAR) WITH SNP rs_7927894 *FLG*, rs_11466749 *TSLP* AND rs_7216389 *ORMDL3*

The evidence is obtained of a statistically significant increased risk by 4.85 fold within C/T rs_7927894 FLG genotype carriers (OR = 4.85 (95% CI 1.16, 20.27), rc = 0.245) and decreased risk trending to significance by 5.88 fold A/G rs_11466749 TSLP genotype carriers (OR = 0.17 (95% CI 0.02, 1.38), rc = −0.196) with developing the aforesaid AD phenotype (Table 5).

Summarizing the dataset obtained, genotypes with the significant risk and candidiate genotypes with a trend to significance of developing the different AD phenotypes are provided with the respective OR (Fig. 1).

Tab. 5 Associations and frequency of SNP rs_7927894 *FLG*, rs_11466749 *TSLP* and rs_7216389 *ORMDL3* with AD combined with BA (possibly, SARC and/or PAR) phenotype in children.

Cohorts	Genotypes SNP rs_7927894 *FLG*		
	C/C	C/T	T/T
Main group	30.0%	70.0%	0.0%
Control group	47.5%	32.5%	20.0%
Statistical significance by FET, two-tailed	p > 0.05	p < 0.05	p > 0.05
OR (95% CI)		4.85 (1.16; 20.27)	
PCC (rc)		0.245	
	Genotypes SNP rs_11466749 *TSLP*		
	A/A	A/G	G/G
Main group	80.0%	10.0%	10.0%
Control group	56.3%	40.0%	3.7%
Statistical significance by FET, two-tailed	p > 0.05	p = 0.0806	p > 0.05
OR (95% CI)		0.17 (0.02; 1.38)	
PCC (rc)		−0.196	
	Genotypes SNP rs_7216389 *ORMDL3*		
	C/C	C/T	T/T
Main group	30.0%	50.0%	20.0%
Control group	30.0%	55.0%	15.0%
Statistical significance by FET, two-tailed	p > 0.05	p > 0.05	p > 0.05

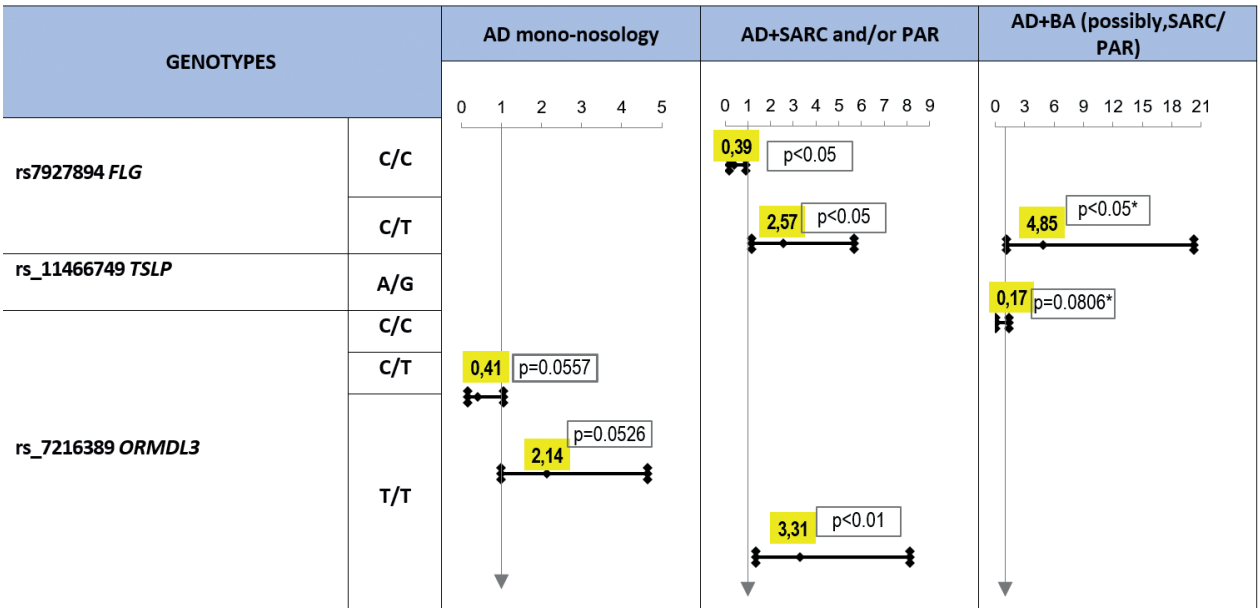

Fig. 1 Risk (OR (95% CI)) of different AD phenotypes' development within different genotypes of SNP rs_7927894 *FLG*, rs_11466749 *TSLP*, rs_7216389 *ORMDL3* (significance by Pearson's χ2-test (* by FET, two-tailed).

DISCUSSION

Significant association by 2.57 fold ($p < 0.05$) with developing AD combined with SARC and/or PAR phenotype was detected with the carriage of genotype C/T rs_7927894 *FLG* and by 3.31 fold ($p < 0.01$) with genotype T/T rs_7216389 *ORMDL3* (PCC between 0.217 and 0.246 ($p < 0.05$)). Along with that, carriage of the genotype C/C rs_7927894 *FLG* does significantly decrease the risk of the mentioned AD phenotype by 2.56 fold (OR = 0.39; $p < 0.05$). This suggests a novel approach towards the genetic background of AD phenotypes compared to prevailing studies on FLG null loss-of function mutations (6, 13).

Statistically significant association of AD combined with BA (possibly, SARC and/or PAR) phenotype with the carriage of genotype C/T rs_7927894 *FLG* was detected with the increased risk (OR = 4.85; $p < 0.05$) which is even higher than in relevant studies of *FLG* gene variants' associations with the risk of developing AD with BA (7, 14).

Results obtained which need to be confirmed in further studies – are the genotypes' s trending to significance associations with the risks of developing different AD phenotypes (p-value between 0.05 and 0.1). Thus, AD alone phenotype is by 2.44 fold less likely to develop within the carriers of genotype C/C rs_7216389 *ORMDL3* (OR = 0.41) and by 2.14 fold more likely to develop within the carriers of genotype C/T rs_7216389 *ORMDL3* (OR = 2.14). Result obtained for A/G rs_11466749 variant of *TSLP* gene – which had been found in association with AD development in recent studies (7, 14) – is that AD combined with BA (possibly, SARC and/or PAR) is by 5.88 fold less likely to develop within it' s carriers (OR = 0.17). This paves the way for further studies of AD phenotypes' genetics.

CONCLUSIONS

AD phenotypes' development is significantly associated with the genotypes C/T rs_7927894 of *FLG* gene and T/T rs_7216389 of *ORMDL3* gene.

Children carrying genotype C/T rs_7927894 of *FLG* gene are exposed to a significantly by 2.57 fold increased risk of developing AD combined with SARC and/or PAR and significantly by 4.85 fold increased risk of developing AD combined with BA (possibly, SARC and/or PAR) phenotypes.

Children carrying genotype T/T rs_7216389 of *ORMDL3* gene are exposed to the significantly by 3.31 fold increased risk of developing AD combined with SARC and/or PAR phenotype.

Children carrying genotype C/C rs_7927894 of *FLG* gene have the significantly by 2.56 fold decreased risk of developing AD combined with SARC and/or PAR phenotype.

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