

# Association between single nucleotide polymorphisms of interleukin-35 genes and atopic dermatitis

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## Abstract

**Introduction:** The pathogenesis of atopic dermatitis (AD) involves complex interactions between environmental factors, the skin microbiome, epidermal barrier defects, and altered immune responses that develop on a not fully understood specific genetic background.

**Aim:** We aimed to evaluate the contribution of single nucleotide polymorphisms (SNPs) in the IL-35 genes (*IL-12A* and *EBI3*) towards AD susceptibility and clinical characteristics of AD in the Polish population. Two SNPs (rs568408, rs582054) in *IL-12A* and one SNP (rs428253) in *EBI3* were selected.

**Material and methods:** Blood samples were collected from 202 AD patients and 178 healthy individuals. SNPs in IL-35 genes were analysed by the polymerase chain reaction with sequence-specific primers (SSP-PCR) method.

**Results:** For *IL-12A* rs568408, the AA genotype was significantly linked to increased odds of AD (OR = 34.61; 95% CI: 2.06–579.97,  $p = 0.0137$ ) and marginally associated with normal total serum IgE levels (OR = 2.82; 95% CI: 0.97–8.16;  $p = 0.05$ ), while the GA genotype showed significantly reduced odds of AD (OR = 0.53; 95% CI: 0.34–0.81;  $p = 0.0035$ ). In the context of *IL-12A* rs582054, TT genotype carriers had increased odds of AD (OR = 2.05; 95% CI: 1.08–3.85;  $p = 0.03$ ). Patients with the GG genotype of *EBI3* rs428253 had decreased odds of high total serum IgE levels (OR = 0.42; 95% CI: 0.20–0.86;  $p = 0.02$ ) and milder pruritus severity compared to CC genotype carriers (4.12 vs. 7.50;  $p = 0.02$ ).

**Conclusions:** IL-35 genetic variations appear to play a role in AD pathogenesis.

**Key words:** atopic dermatitis, interleukin-35, single nucleotide polymorphisms.

## Introduction

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease characterized by highly heterogeneous clinical manifestations, including skin manifestations, longitudinal course, comorbidities, and treatment outcomes [1]. AD is also heterogeneous from the point of view of pathogenesis, which involves complex interactions between environmental factors, the skin microbiome, epidermal barrier defects, and altered immune responses that develop on a still incompletely understood specific genetic and epigenetic background [2]. The main groups of genes associated with AD pathogenesis include epidermal barrier genes, genes involved in innate and adaptive immune mechanisms, and genes encoding alarmins produced by keratinocytes [3]. Loss-of-function mutations in the gene encoding filaggrin (FLG) remain the strongest identified genetic risk factors for AD, associated with severe course, early onset of the disease, and elevated levels of immunoglobulin E (IgE) [4–6].

The strong genetic predisposition characterizing AD prompts detailed and intensive research in that area. In our previous work, we investigated the gene expression of interleukin (IL)-35 in lesional and non-lesional AD skin compared to the skin of healthy controls [7]. We found that the lesional skin of AD patients is characterized by significantly higher expression levels of IL-35 compared to healthy skin. Additionally, the expression level of IL-35 in both lesional and non-lesional skin significantly negatively correlated with AD severity, suggesting a potential role of this cytokine in AD pathogenesis [7]. IL-35 is a cytokine with interesting immunomodulatory properties belonging to the IL-12 family, which also contains IL-12, IL-23, and IL-27 [8]. Unlike other family members, IL-35 is considered a strictly anti-inflammatory cytokine that maintains immune tolerance [8]. The immunomodulatory properties of IL-35 include inducing a unique population of regulatory T cells releasing IL-35, termed iTr35, as well as IL-10-producing regulatory B cells (IL-10+Bregs) and

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IL-35-producing regulatory B cells (IL-35+Bregs), while suppressing Th1, Th17, and Th2 cell responses [8].

Like autoimmune disorders, it is hypothesized that in allergic conditions such as AD, regulatory T cells fail to adequately suppress immune responses, allowing immune polarization, which in the context of AD is typically skewed toward a Th2-polarized immune profile [2]. In addition to the Th2 response, other immune cells such as Th22, Th17, and Th1 cells have been shown to play a role in the pathogenesis of AD, and their involvement can vary based on the chronicity of the disease, age, and ethnic background of the patient [9]. Two studies on serum levels of IL-35 in AD patients showed conflicting results: one found increased levels in AD infants [10], while the other found decreased levels in AD individuals compared to healthy controls [11]. However, the study groups from the two aforementioned studies varied in terms of age and ethnicity, which may potentially explain the observed discrepancies in the results. Although the role of IL-35 in the pathogenesis of AD remains enigmatic, considering the function of IL-35, it appears to be potentially significant in the development of AD. This heterodimeric cytokine is composed of p35 and EBI3 subunits, which are encoded by two separate genes called *IL12A* and *EBI3*, respectively [8]. The *IL-12A* gene is located on the 3p12-13.2 chromosome and encodes the p35 subunit, which is shared between IL-12 and IL-35 [12]. The *EBI3* gene, which encodes the EBI3 subunit that is part of IL-27 and IL-35 [12], is situated on chromosome 19p13.3, close to loci 19p13.2 that have been identified as risk loci for AD [13]. Interestingly, evidence suggests that the p35 and EBI3 subunits may act as a homodimer. The p35 subunit may exhibit some of the immune-regulatory properties of the heterodimeric IL-35 cytokine [14], while the EBI3 subunit may help maintain Th1 and Th2 response balance [15].

**Table 1.** Demographic and clinical characteristics of study participants

Characteristic	AD patients (n = 202)	Healthy controls (n = 178)
Sex, n (%):		
Female	117 (57.92)	91 (51.12)
Male	85 (42.08)	86 (48.31)*
Age, mean ± SD	18.78 ±12.77	29.95 ±11.21
SCORAD, mean ± SD	49.04 ±23.6	–
Pruritus severity, mean ± SD	5.35 ±3.16	–
Total IgE serum level, mean ± SD	1539.1 ±1934.39 kU/l	–
Age of disease onset, mean ± SD	5.2 ±14.14	–
Asthma, n (%)	11 (18.97)**	–

AD – atopic dermatitis, SD – standard deviation. \*Missing data for 1 person. \*\*Data on the coexistence of asthma was available only in 58 patients; percent-age data were calculated assuming n = 58 in this case.

## Aim

These observations encouraged us to evaluate the contribution of single nucleotide polymorphisms (SNPs) in the IL-35 genes (*IL-12A* and *EBI3*) towards AD susceptibility and clinical characteristics of AD in the Polish population. Two SNPs (*rs568408*, *rs582054*) in *IL-12A* and one SNP (*rs428253*) in *EBI3* were selected to analyse the association of IL-35 gene polymorphism and AD. SNPs were chosen based on their previously documented significance in other atopic diseases, their functional relevance, and the minor allele frequency (MAF) ≥0.1 in the Caucasian population [16–19].

## Material and methods

The study included 202 patients with AD recruited from the Department of Dermatology, Venereology, and Allergology at the Medical University of Gdansk, based on AD diagnosis criteria proposed by Hanifin and Rajka [20] and 178 healthy individuals with no medical history of allergy, autoimmune diseases, or malignancies. Patients receiving immunosuppressive treatment or other immunotherapies were excluded from the study. Both AD patients and healthy individuals were Caucasian. The patient group consisted of 117 (57.92%) females and 85 (42.08%) males with a mean age of 18.78 ±12.77 years. The mean SCORAD score, and pruritus severity were 49.04 ±23.6 and 5.35 ±3.16, respectively. The mean age of disease onset was 5.2 ±14.14. The mean level of the total serum IgE was 1539.1 ±1934.39 kU/l. For the coexistence of asthma, we had data for 58 patients with AD, and asthma was present in 11 out of 58 patients. The healthy control group comprised 91 (51.12%) females and 86 (48.31%) males; missing data for 1 person, with a mean age of 29.95±11.21. Both groups were sex-matched ( $p = 0.2$ ) but not age-matched ( $p = 0.001$ ).

The clinical characteristics of the study group are presented in Table 1.

### Determination of atopic dermatitis severity

AD severity was assessed by the SCORAD (Severity Scoring of Atopic Dermatitis) scale, in which AD severity is defined as mild (SCORAD < 25), moderate (SCORAD 25–50), and severe (SCORAD > 50) [21].

Pruritus severity was estimated using the visual analogue scale (VAS: 0–3 – mild pruritus, > 3–7 – moderate pruritus, > 7–9 – severe pruritus, > 9 – very severe pruritus).

### Determination of the total serum IgE level

Total serum IgE levels were measured by the fluorescent enzyme immunoassay using the Uni-CAP 100 System (Phadia, Sweden) according to the manufacturer's protocol. A normal total serum IgE level was defined as a total serum IgE level of ≤ 100 kU/l, while an elevated

total serum IgE level was defined as a total serum IgE level of > 100 kU/l.

**Genotyping**

Genomic DNA was isolated from peripheral blood samples of both AD patients and healthy individuals using the Blood Mini Kit from A&A Biotechnology (Gdansk, Poland), following the manufacturer’s protocol. SNPs in both IL-35 genes: *IL-12A* (rs568408, rs582054) and *EBI3* (rs428253) were analysed by the polymerase chain reaction with sequence-specific primers (SSP-PCR) method (Table 2).

**Statistical analysis**

Statistical analyses were performed using the Statistica 12.0 software package (StatSoft, Inc. 2015, Tulsa, OK,

USA). Before making comparisons, the Shapiro-Wilk test was used to check the normality of the analysed measurable variables. Continuous and categorical variables were analysed by *t*-Student’s test, Mann-Whitney *U* test, Kruskal-Wallis, and  $\chi^2$  or Fisher test as appropriate.

A logistic regression model was used to calculate the odds ratio (OR) and 95% confidence intervals (CIs). *P* < 0.05 was considered statistically significant.

**Results**

***IL-12A* rs568408**

For *IL-12A* rs568408 polymorphism, the frequency of the AA genotype was significantly higher in AD patients than in the healthy control group (8.63% vs. 0.0%; odds

**Table 2.** PCR primers and conditions

Gene	Reference SNP	Exon/ intron	Primer sequence	Tm [°C]	PCR products
<i>IL12A</i>	rs568408	3’UTR	Forward inner: GAAGGATGGGACTATTACATCCACCTA	63	Fo-Ri (GG): 391 bp Fi-Ro (AA): 271 bp Fo-Ro: 605 bp
			Reverse inner: AAATGTCAAAAATACTTGATCAGAGGTCTC	63	
			Forward outer: CATGTACTGGCTTCACTCATTTTTATGA	63	
			Reverse outer: AGTTAGCTCAGATGCTTTCATGATTACC	63	
	rs582054	Intron	Forward inner: TTTACTCTCTTAATGTGGGTGTCGGAA	67	Fo-Ri (TT): 353 bp Fi-Ro (AA): 252 bp Fo-Ro: 551 bp
			Reverse inner: GGAAGGGAAGCAAAGCTTCTAGTGA	67	
			Forward outer: TTGAGGACACTGCTTACTGGATTAC	67	
			Reverse outer: TTACGATCATTTTTCCATTCAACAGCCT	67	
<i>EBI3</i>	rs428253	Intron	Forward inner: GCGAATTTGAGTCACTCATTCTATC	66	Fo-Ri (GG): 345 bp Fi-Ro (CC): 245 bp Fo-Ro: 532 bp
			Reverse inner: TGTCTCAAAAACAAAACAAAAGAATCC	66	
			Forward outer: CTGCAGTGGAAAGGAAAGGTATGTGG	66	
			Reverse outer: GGCCAACATGGTAAAACCCTATCTACT	66	

Fo – forward outer primer, Ro – reverse outer primer, Fi – forward inner primer, Ri – reverse inner primer, bp – base pair.

**Table 3.** Distribution of *IL-12A* rs568408, *IL-12A* rs582054, and *EBI3* rs428253 genotypic frequencies among AD patients and healthy controls

Genotypes	AD patients (n = 202)	Healthy controls (n = 178)	P-value	OR (95% CI)
<i>IL-12A</i> rs568408, n (%):				
GG	67 (34.01)	50 (28.09)	0.22	–
GA	113 (57.36)	128 (71.91)	0.003	0.53 (0.34–0.81), <i>p</i> = 0.0035
AA	17 (8.63) <sup>1</sup>	0 (0.00) <sup>1</sup>	0.00006	34.61 (2.06–579.97), <i>p</i> = 0.0137
<i>IL-12A</i> rs582054, n (%):				
AT	75 (37.13)	77 (43.26)	0.22	–
AA	93 (46.04)	85 (47.75)	0.74	–
TT	34 (16.83)	16 (8.99)	0.02	2.05 (1.08–3.85), <i>p</i> = 0.03
<i>EBI3</i> rs428253, n (%):				
CG	85 (42.08)	60 (33.71)	0.09	–
GG	76 (37.62)	83 (46.63)	0.07	–
CC	41 (20.30)	35 (19.66)	0.87	–

AD – atopic dermatitis, OR – odds ratio, CI – confidence interval. <sup>1</sup>Percentage data were calculated assuming n = 197 and n = 178 in the case of AD patients and healthy controls, respectively.

ratio [OR] = 34.61; 95% CI: 2.06–579.97;  $p = 0.0137$ ), indicating a potential association of this genotype with increased susceptibility to AD. The GA genotype of *IL-12A* (rs568408) was significantly less frequent in AD patients when compared to the healthy control group (57.36% vs. 71.91%; OR = 0.53; 95% CI: 0.34–0.81;  $p = 0.0035$ ). No significant differences in the occurrence of the GG genotype of *IL-12A* rs568408 between AD patients and the healthy control group were found ( $p = 0.22$ ) (Table 3). Furthermore, there were no significant differences in allele frequency distribution for the *IL-12A* rs568408 between AD patients and the healthy control group ( $p = 0.7$ ) (Table 4).

The results showed that patients carrying the AA genotype of *IL-12A* rs568408 had significantly lower total serum IgE levels than patients carrying the GA (611.98 kU/l vs. 1707.18 kU/l;  $p = 0.01$ ) or GG genotype (611.98 kU/l vs. 1608.44 kU/l;  $p = 0.02$ ). There was no significant difference in levels of serum total IgE between patients carrying the GG and GA genotypes (1608.44 kU/l vs. 1707.18 kU/l;  $p = 1.0$ ).

When AD patients were subdivided into two populations with normal total serum IgE levels and elevated total serum IgE levels, the frequency of the occurrence of the GG or GA genotype *IL-12A* s568408 did not differ significantly between both groups (25.64% vs. 32.00%,  $p = 0.45$  or 56.41% vs. 60.80%,  $p = 0.63$ , respectively). In contrast, the AA genotype was significantly more common in patients with normal total serum IgE levels compared to patients with elevated total serum IgE levels (17.95% vs. 7.20%,  $p = 0.048$ ). Furthermore, the presence of the AA genotype was marginally significantly associated with increased odds of normal total serum IgE levels (OR = 2.82; 95% CI: 0.97–8.16;  $p = 0.05$ ).

There was no association between *IL-12A* rs568408 and pruritus ( $p = 0.40$ ), the mean SCORAD score ( $p = 0.60$ ), or the onset age of the disease ( $p = 0.45$ ).

Moreover, there were no significant differences in the frequency of the GG, GA, or AA genotype of *IL-12A*

s568408 between AD patients with concomitant asthma and patients without asthma (30.0% vs. 25.53%;  $p = 0.92$ , 70.0% vs. 59.57%;  $p = 0.80$ , or 0.0% vs. 14.89%;  $p = 0.44$ , respectively).

***IL-12A* rs582054**

For *IL-12A* rs582054 polymorphism, only the TT genotype showed significant differences in the frequency distribution between AD patients and healthy control groups, with the TT genotype being more prevalent in the AD group (16.83% vs. 8.99%; OR = 2.05; 95% CI: 1.08–3.85;  $p = 0.03$ ) (Table 3). Moreover, we found no significant differences in the allele frequency distribution for the *IL-12A* rs582054 ( $p = 0.16$ ) (Table 4).

Patients carrying the AA genotype of *IL-12A* rs582054 presented lower levels of serum total IgE than patients carrying the AT (929.15 kU/l vs. 1894.22 kU/l;  $p = 0.003$ ) or TT genotype (929.15 kU/l vs. 2581.85 kU/l;  $p = 0.002$ ). There was no significant difference in levels of serum total IgE between patients carrying the AT and TT genotypes (1894.22 kU/l vs. 2581.85 kU/l;  $p = 1.0$ ).

When AD patients were subdivided into two populations with normal total serum IgE levels and elevated total serum IgE levels, the frequency of the AA or TT genotype of *IL-12A* rs582054 did not differ significantly between both groups (60.0% vs. 43.41%;  $p = 0.07$  or 15.0% vs. 14.73%;  $p = 0.96$ ). The AT genotype showed a marginally significant difference in occurrence between both groups, being more prevalent in patients with elevated total serum IgE levels (41.86% vs. 25.0%;  $p = 0.05$ ). Furthermore, the AT genotype was slightly associated with increased odds of elevated total serum IgE levels (OR = 2.16; 95% CI: 0.97–4.79;  $p = 0.058$ ).

There was no association between *IL-12A* rs582054 and pruritus ( $p = 0.69$ ), the mean SCORAD score ( $p = 0.39$ ), or the onset age of the disease ( $p = 0.88$ ).

There were no significant differences in the frequency of the AT or AA genotype of *IL-12A* rs582054 between

**Table 4.** Distribution of *IL-12A* rs568408, *IL-12A* rs582054, and *EBI3* rs428253 allelic frequencies among AD patients and healthy controls

Allele	AD patients (2 N = 404)	Healthy controls (2 N = 356)	P-value
<i>IL-12A</i> rs568408, n (%):			
Allele G	247 (62.69)	228 (64.04)	
Allele A	147 (37.31) <sup>1</sup>	128 (35.96) <sup>1</sup>	0.7
<i>IL-12A</i> rs582054, n (%):			
Allele A	261 (64.60)	247 (69.38)	
Allele T	143 (35.40)	109 (30.62)	0.16
<i>EBI3</i> rs428253, n (%):			
Allele G	237 (58.66)	226 (63.48)	
Allele C	167 (41.34)	130 (36.52)	0.17

AD – atopic dermatitis. <sup>1</sup>Percentage data were calculated assuming N = 394 and N = 356 in the case of AD patients and healthy controls, respectively.

patients with AD and concomitant asthma and patients without asthma (63.64% vs. 55.32%;  $p = 0.62$  or 36.36% vs. 17.02%;  $p = 0.15$ , respectively). The TT genotype was significantly less common in AD patients and concomitant asthma compared to AD patients without asthma (0.0% vs. 27.66%;  $p = 0.047$ ; OR = 0.11; 95% CI: 0.0061–2.02;  $p = 0.14$ ).

**EBI3 rs428253**

For the *EBI3* rs428253 polymorphism, no significant differences were found in the frequency distribution of either genotypes or alleles between patients with AD and the healthy control group (Tables 3, 4).

However, we found that patients carrying the GG genotype of *EBI3* rs428253 had significantly lower levels of serum total IgE than patients carrying the CG genotype (1103.89 kU/l vs. 1958.95 kU/l;  $p = 0.004$ ). There were no significant differences in levels of serum total IgE between patients carrying the CG and CC genotypes or between patients carrying the CC and GG genotypes (1958.95 kU/l vs. 1381.64 kU/l;  $p = 0.76$  or 1381.64 kU/l vs. 1103.89 kU/l;  $p = 0.34$ , respectively).

When AD patients were subdivided into two populations with normal total serum IgE levels and elevated total serum IgE levels, the frequency of the CG or CC genotype of *EBI3* rs428253 did not differ significantly between both groups (32.50% vs. 47.29%;  $p = 0.10$  or 17.50% vs. 23.26%;  $p = 0.44$ , respectively). The GG genotype was significantly more common in the group of AD patients with normal total serum IgE levels compared to AD patients with elevated total serum IgE levels (50.0% vs. 29.46%;

$p = 0.02$ ). Patients carrying the GG genotype were significantly less likely to have elevated total serum IgE levels than patients without this genotype (OR = 0.42; 95% CI: 0.20–0.86;  $p = 0.02$ ).

Moreover, we found an association between *EBI3* rs428253 and pruritus severity ( $p = 0.01$ ). Patients carrying the GG genotype were characterized by significantly milder severity of pruritus compared to patients carrying the CC genotype (4.12 vs. 7.50;  $p = 0.02$ ). No significant differences in pruritus severity were found between patients carrying the CG and GG genotypes or between patients carrying the CG and CC genotypes (5.85 vs. 4.12;  $p = 0.13$  or 5.85 vs. 7.50;  $p = 0.54$ , respectively).

There was no association between *EBI3* rs428253 and the mean SCORAD score ( $p = 0.13$ ), or the onset age of the disease ( $p = 0.15$ ). Moreover, we found no significant differences in the frequency of the CG, GG, or CC genotypes of *EBI3* rs428253 between patients with AD and concomitant asthma and patients without asthma (63.64% vs. 40.43%;  $p = 0.16$ , 27.27% vs. 46.81%;  $p = 0.24$ , or 9.09% vs. 12.77%;  $p = 0.74$ , respectively).

The association of *IL-12A* and *EBI3* polymorphisms with clinical characteristics of AD is presented in Table 5.

**Discussion**

To discuss the association of SNPs in the IL-35 genes with AD, we analysed the relationship between *IL-12A* rs568408, *IL-12A* rs582054, and *EBI3* rs428253 polymorphisms and AD susceptibility in the Polish population, as well as the clinical characteristics of AD, including disease severity, pruritus severity, age of disease onset,

**Table 5.** Association of *IL-12A* and *EBI3* polymorphisms with clinical characteristics of AD, including disease severity, pruritus severity, age of disease onset, and serum total IgE levels

Polymorphisms	SCORAD, mean ± SD	Pruritus severity, mean ± SD	Age of disease onset, mean ± SD	Serum total IgE levels, mean ± SD
<i>IL-12A</i> rs568408:				
GG	48.53 ±23.83	5.93 ±3.43	5.47 ±8.30	1608.44 ±1818.74
GA	50.53 ±23.52	5.27 ±3.18	5.70 ±17.52	1707.18 ±2071.80
AA	41.80 ±27.43	4.00 ±2.65	2.43 ±3.36	611.98 ±1318.87
	$p = 0.6$	$p = 0.4$	$p = 0.45$	$p = 0.01$
<i>IL-12A</i> rs582054:				
AT	52.20 ±23.56	5.71 ±3.03	6.23 ±17.47	1894.22 ±2086.91
AA	47.06 ±24.24	5.00 ±3.76	2.01 ±1.56	929.15 ±1513.23
TT	42.44 ±23.24	4.77 ±3.14	5.62 ±10.85	2581.85 ±2124.64
	$p = 0.39$	$p = 0.69$	$p = 0.88$	$p = 0.003$
<i>EBI3</i> rs428253:				
CG	52.91 ±21.77	5.85 ±2.94	5.35 ±19.53	1958.95 ±2099.85
GG	42.52 ±24.13	4.12 ±3.26	3.70 ±4.81	1103.89 ±1728.08
CC	57.03 ±24.81	7.50 ±2.39	9.75 ±15.23	1381.64 ±1760.54
	$p = 0.13$	$p = 0.01$	$p = 0.15$	$p = 0.006$

serum total IgE levels, and the presence of coexisting asthma.

To the best of our knowledge, no studies have reported an analysis in which both IL-35 genes (*IL12A* and *EBI3*) have been analysed for AD.

For *IL-12A* rs568408, we found that patients carrying the AA genotype have nearly 35-fold increased odds of developing AD when compared to patients without this genotype, suggesting that this genotype contributes to AD susceptibility. On the other hand, patients harbouring the GA genotype were significantly less likely to have AD when compared to patients without this genotype. Hence, it can be postulated that the GA genotype may exhibit a protective effect against the onset of AD. As far as we know, the *IL-12A* rs568408 polymorphism has not been previously investigated in AD patients, and the contribution of *IL-12A* rs568408 to AD is largely unknown. However, the *IL-12A* rs568408 polymorphism has been previously linked to the pathogenesis and clinical manifestations of another Th2 cell-mediated disorder, such as asthma, in the Taiwanese population [16]. In this investigation, the AA genotype of *IL-12A* rs568408 was significantly associated with increased susceptibility to asthma, while the presence of the A allele at this locus was associated with heightened symptom severity in affected individuals [16]. In contrast, none of the patients in our study with AD and concomitant asthma were found to carry the AA genotype of *IL-12A* rs568408. However, the Taiwanese population has a distinct genetic background and is exposed to, among other things, different environmental factors compared to our population.

Furthermore, in our study, patients with the AA genotype *IL-12A* rs568408 have nearly 3-fold increased odds of normal total IgE levels, suggesting that this genotype may predispose to AD development with normal total IgE levels. The *IL-12A* gene encodes the p35 subunit which is shared between IL-12 and IL-35 [12]. IL-12 is a cytokine that primarily promotes Th1 responses while suppressing Th2 responses [22]. IL-35 is believed to inhibit Th1, Th2, and Th17 cells while promoting regulatory T-cell development [23]. Additionally, both cytokines can influence IgE levels. IL-12 by suppressing Th2 responses, leads to a shift in the immune response away from IgE production [22]. IL-35 has been shown to also have the ability to suppress the production of both allergen-specific IgE and total IgE production [24, 25]. Therefore, SNPs within the *IL-12A* gene may have the potential to influence the expression of both IL-12 and IL-35, shaping the immune response and resulting in changes in AD susceptibility and IgE production. Intriguingly, the GA genotype of *IL-12A* rs568408 had a protective effect with an OR of 0.53 against AD development, but at the same time, patients with this genotype had significantly high total IgE levels. These seemingly contradictory results can be explained, among others, by the complexity of the pathogenesis of AD [26]. The GA genotype of *IL-12A* rs568408 may impact

IgE levels through different pathways that are not directly related to the development of AD. Although high IgE levels are a common feature of AD, present in 80% of patients [27], it is not the only factor associated with the disease. Other factors, such as environmental, genetic, and immunological factors, as well as skin barrier defects and the skin microbiome, which interact in a complex manner, also play a role in the development of AD [26]. On the other hand, the GA genotype of *IL-12A* rs568408 may be associated with other genetic factors or pathways that protect against the development of AD, independent of IgE levels. Moreover, it is important to consider that genetic variants can have pleiotropic effects, meaning that one genetic variant can influence multiple traits via independent biological pathways; genetic variants may have the opposite effect on two traits [28].

In the context of *IL-12A* rs582054, our research indicates that individuals with the TT genotype have 2-fold increased odds of developing AD; however, interestingly, this genotype was notably less prevalent in AD patients with coexisting asthma compared to those without asthma. Furthermore, the AT genotype showed a marginally significant association with elevated total serum IgE levels in AD patients, with an OR of 2.16. Like us, Namkung *et al.* investigated the relationship of *IL-12A* rs582054 with AD in Koreans, finding a particular association with extrinsic AD, which refers to elevated serum IgE levels [18].

For *EBI3* rs428253, although no differences in the distribution of either allelic or genotypic frequencies were found in our study, the GG genotype showed a protective effect with an OR of 0.42 against elevated total serum IgE levels in AD patients as compared to those without this genotype. Moreover, patients carrying the GG genotype experienced significantly less severe pruritus compared to patients carrying the CC genotype. The less severe pruritus in patients with the GG genotype can be attributed to the lower disease severity observed in these patients compared to those with the CG or CC genotype. However, differences in disease severity between these patients were not statistically significant. To date, there has been no research addressing the relationship between SNPs in the *EBI3* gene and AD or IgE levels. Zhang *et al.* discovered that the CG/CC genotype of *EBI3* rs428253 is significantly associated with a protective effect against allergic rhinitis in the Chinese population [19]. Interestingly, findings from murine models suggest that *EBI3* protein deficiency shifts the balance towards Th2-mediated inflammatory reactions while attenuating Th1-mediated responses [15]. Moreover, *EBI3* protein deficiency resulted in an elevation in IgE levels [15]. Therefore, it appears that *EBI3* may possess a protective effect against Th2-mediated inflammation also on the protein level.

As for the limitations, our study included a relatively small number of AD patients and controls, which may lead to false positive or negative findings due to lower statistical analysis power. However, our observations

shed light on new SNPs that may be associated with AD. Moreover, it should be noted that we lacked data on the coexistence of asthma in a certain number of our patients with AD and the analysed data represent a group of 57/202 patients with AD, which may affect these results. Additionally, we did not assess the serum levels of IL-35 in our study participants, which limited our ability to analyse the genotype-phenotype correlation. Investigating this correlation in future studies would be valuable.

## Conclusions

Our findings suggest that IL-35 genetic variations may play a role in AD pathogenesis. The GA genotype of *IL-12A* rs568408 may have a protective effect against AD development, while the AA genotype of *IL-12A* rs568408 appears to predispose individuals to AD development with normal total IgE levels. In the context of *IL-12A* rs582054, individuals with the TT genotype may be more susceptible to developing AD, while the AT genotype may contribute to elevated total serum IgE levels. *EBI3* rs428253 does not appear to influence susceptibility to AD, but the GG genotype may have a protective effect against elevated total serum IgE levels. To confirm these assumptions further studies are warranted.

Overall, these findings underscore the complexity of genetic influences on immune-mediated diseases like AD and highlight the need for further investigation to understand the specific mechanisms underlying these associations. The complex and not fully elucidated pathogenesis of AD shapes different endotypes and phenotypes of the disease, posing challenges in the therapeutic and diagnostic aspects [27]. Understanding this complex disease in its full phenotypic and mechanistic spectrum is essential to the precision medicine approach, to which we are currently pursuing. Furthermore, an early and precise identification of the genetic risk factors for developing AD, as well as the risk of experiencing an atopic march, holds great promise for a disease-modifying prevention strategy from early life onwards.

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## Ethical approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Independent Bioethics Committee for Scientific Research at

the Medical University of Gdansk (KB/679/2023). Written consent was obtained from all patients before enrolment.

## Conflict of interest

The authors declare no conflict of interest.

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