

The A/A genotype of an interleukin-17A polymorphism predisposes to increased severity of atopic dermatitis and coexistence with asthma

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Summary

Introduction. Studies have found that the interleukin-23/T helper 17 (IL-23/Th17) pathway plays an important role in the pathogenesis of atopic dermatitis (AD). Inhibition of the IL-23/Th17 pathway with monoclonal antibodies reduces skin inflammation in animal models.

Aim. To investigate the association between IL-17A and IL-23R gene single nucleotide polymorphisms (SNPs) and the development of AD in a Polish population.

Methods. Blood samples were collected from 166 patients with AD and 160 controls. We analyzed two SNPs, –152 G/A IL-17A and 1142 G/A IL-23R, using PCR and restriction fragment length polymorphism (RFLP) analysis.

Results. There was no statistically significant difference between the examined IL-17A SNP and the incidence of AD ($P > 0.05$ for all comparisons). Analysis of the IL-23R gene SNP showed no relationship between AD and the G/A genotype or presence of the A allele. The study did not establish any links between the IL-23R and IL-17A gene SNPs and the likelihood of developing AD resulting from gene–gene interaction. However, there was a significant relationship between the A/A genotype in the –152 G/A IL-17A SNP and the coexistence of AD and asthma ($P < 0.04$). Analyzing the association between AD severity and the occurrence of IL-17A SNP, we found that subjects with the A/A genotype were at higher risk of developing moderate or severe AD ($P = 0.02$).

Conclusions. We found no evidence of any effect of IL-17A or IL-23R SNPs on the occurrence of AD in our Polish population. However, the A/A genotype in IL-17A was found to predispose to increased AD severity and coexistence of AD and asthma.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease, occurring in 10–20% of children, and is often associated with other systemic atopic disorders.¹ Skin

barrier dysfunction and immune disturbances are considered to be the key elements in the development and progression of AD.^{2,3} Recently, the T helper (Th)1/Th2 concept of autoimmunity and allergy has been revisited, with a role suggested for IL-17 produced by Th17 cells.⁶ IL-17 is involved in a range of biological processes *in vivo*, which are critical for many diseases with an inflammatory component.^{4,5} The possible role of IL-17 in cutaneous remodelling in AD may be mediated by its ability to stimulate epithelial cells and fibroblasts to secrete proinflammatory cytokines such as IL-6, IL-8 and IL-11. The stimulatory effects of IL-17

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could promote tissue fibrosis and the development of chronic inflammatory cutaneous lesions.⁵

Phenotypic analysis of peripheral blood mononuclear cells (PBMCs) from patients with AD showed a marked increase in the IL-17+ CD4+ T-cell population compared with PBMCs from healthy controls. The highest percentage of IL-17-producing cells was found in patients with severe AD, suggesting a direct correlation between the presence of Th17 cells and the severity of the disease.⁵

IL-23 is a cytokine required for the Th17-mediated autoimmune response, and it affects a number of chronic immune disorders. The IL-23 receptor (IL-23R) is encoded by the *IL-23R* gene, which is located on chromosome 1p31.3. IL-23R is expressed by dendritic cells, macrophages and natural killer cells, and by CD4+ and CD8+ T cells. IL-23R plays a crucial role in the differentiation of T-helper lymphocytes into Th17 cells. Inhibition of the IL-23/Th17 pathway reduces skin inflammation in animal models.^{6,7} In recent years, there has been an increasing number of reports on the significance of IL-23R in susceptibility to chronic inflammatory diseases.^{6,7} Nevertheless, there is still a marked paucity of data on IL-17 and IL-23 gene SNPs in AD. Hence, the aim of this study was to investigate the association between the IL-17A and IL-23R gene SNPs and the development of AD in a Polish population.

Methods

The study was approved by the local ethics committee of the Medical University of Lodz (ref. no: RNN/208/06/KE), and was conducted in accordance with the Declaration of Helsinki guidelines. All participants

gave their written informed consent before entering the study.

The study enrolled 332 randomly selected participants, who were randomized in a 1 : 1 ratio to the case and control groups (ratio 1 : 1). mean age in the sample was 11.6 years (range 1–42). The case group comprised 166 patients with AD (100 women, 66 men) and a control group of 166 age-matched volunteers (96 women, 70 men). All subjects were exclusively of Eastern European or Polish descent.

Subjects in the control group had no medical history of allergic diseases or familial allergies. Diagnosis of AD was established by two independent dermatologists on the basis of the criteria proposed by Hanifin and Rajka,⁸ and AD severity was assessed using the Rajka and Langeland scale⁹ as mild, moderate or severe. The clinical characteristics of the patients with AD are presented in Table 1.

All the subjects involved in the study were screened for the presence of IL-17A (G/A –152, rs2275913) and IL-23R (G/A 1142, rs11209026) single nucleotide polymorphisms (SNPs). Genomic DNA was prepared from peripheral blood leucocytes using a genomic DNA isolation kit (DNA-Gdansk; II SC, Gdansk, Poland). Analysis of polymorphic variants of the *IL-17A* and *IL-23R* genes was performed by PCR and RFLP (after overnight digestion).^{10,11} The primer sequences of the oligonucleotides and restriction enzymes used are shown in Table 2.

Statistical analysis

We calculated that a minimum of 300 participants would allow us to achieve 80% statistical power with the following assumptions: OR = 2, at least 5% disease

Table 1 Clinical characteristics of patients with AD and control group.

	Patients			Controls		
	Male	Female	Total	Male	Female	Total
Subjects, <i>n</i> (%)	66 (39.76)	100 (60.24)	166 (100)	68 (42.5)	92 (57.5)	160 (100)
Age, years: mean (range)	11.4 (2–40)	11.8 (1–41)	11.6 (1–42)	11.4 (2–40)	11.8 (1–41)	11.6 (100)
AD severity, <i>n</i> (%)						
Mild	35 (21.08)	48 (28.92)	83 (50)	–	–	–
Moderate	31 (18.66)	35 (21.08)	66 (39.76)	–	–	–
Severe	7 (4.21)	10 (6.03)	17 (10.24)	–	–	–
Coexisting disease, <i>n</i> (%)						
Asthma	21 (12.65)	29 (17.47)	50 (30.12)	–	–	–
Allergic rhinitis	30 (18.08)	49 (29.51)	79 (47.59)	–	–	–
Allergic conjunctivitis	14 (8.43)	18 (10.85)	32 (19.28)	–	–	–
Total IgE > 100 U, <i>n</i> (%)	40 (24.1)	49 (29.51)	89 (53.61)	–	–	–

AD, atopic dermatitis.

Table 2 Primer sequences of the oligonucleotides and restriction enzymes.

SNP	PCR product, bp	Restriction enzyme	Restriction fragments	Sequence 5' → 3'
IL-17A -197 G/A	445	<i>Xmn</i> 1	G/G (445); G/A (445 + 288 + 157); A/A (288 + 157)	AGTCACTCTGTGGCCTAAAGTAAAG AGATTTTCTAGTAAACAAGTAAATGA
IL-23R 1142 G/A	350	<i>Hpy</i> 188I	G/G (35 + 65 + 250); G/A (35 + 65 + 250 + 287); A/A (65 + 287)	GCATAACTCTTCTGGCAGCTGTA TGCCACGGTCCAGAAATAC

SNP, single nucleotide polymorphism.

prevalence, complete linkage disequilibrium ($D' = 1$), 1 : 1 case : control ratio and 5% type I error rates for single marker analyses.

The χ^2 test was used to detect significant deviations in genotype frequency from Hardy–Weinberg equilibrium (HWE). OR and 95% CI were calculated by logistic regression. All analyses were performed using Statistica software (v.6.0 Statsoft, Tulsa, OK, USA). $P < 0.05$ was considered statistically significant.

Results

Six controls withdrew from the study, thus statistical analysis was performed for all 166 patients with AD and for the remaining 160 control subjects. Table 3 reports missing data sections for IL-17 in controls ($n = 7$) and for IL-23R in patients ($n = 4$) and controls ($n = 12$).

Genotyping for –157 G/A IL-17A and 1142 G/A IL-23R polymorphisms

Distribution of the genotypes for –157 G/A IL-17A and 1142 G/A IL-23R SNPs was consistent with HWE in both patient and control groups.

Three genotypes (G/G, G/A and A/A) were found in both groups. No significant association between IL-17A SNPs and susceptibility to AD was found ($P > 0.05$). The A/A genotype for the –157 G/A IL-17A SNP was found in 18 patients with AD (11%) and in 10 control subjects (7%). The frequency of the G/A genotype for the IL-17A SNP in patients with AD was 50% ($n = 83$), as opposed to 48% ($n = 74$) in the control group ($P > 0.05$). The incidence of the A/A genotype and of the A allele was statistically higher in patients with AD than in controls ($P = 0.13$ and $P = 0.17$, respectively) (Table 3). We assessed two genotypes, G/G and G/A, for the analyzed IL-23R SNP; the frequency distributions of these two genotypes did not differ between the AD and the control groups.

There was no observable relationship between the occurrence of AD and the presence of the G/A genotype or the A allele (Table 3). The study revealed no correlation between IL-23R and IL-17A gene SNPs and AD risk due to gene–gene interactions (Table 4).

Association between the –197 G/A interleukin-17A polymorphism and asthma, severity of atopic dermatitis and clinical symptoms of atopic dermatitis

Analyzing the association between AD severity and the occurrence of IL-17A SNP, we found that subjects with the A/A genotype were at higher risk of developing moderate or severe AD ($P = 0.02$) (Table 5). We found no association between the presence of the SNP and early onset (before the age of 2 years) of AD ($P > 0.05$).

There was a significant relationship between the A/A genotype and coexistence of AD and asthma in patients ($P < 0.04$) (Table 5). We found a positive correlation between the severity of AD and asthma occurrence ($\chi^2 = 6.79$; $P < 0.01$). No evidence of a link was found between IL-17A SNPs and other atopic diseases, such as rhinitis or conjunctivitis ($P > 0.05$). A search for correlations between the clinical symptoms of AD and the presence of any of the genotypes revealed no association for recurrent itch, skin dryness, keratosis pilaris, neck fold, Dennie–Morgan fold, ichthyosis vulgaris or palmar hyperlinearity, but there was an increased risk (OR = 2.67) of white dermographism in patients with the A/A genotype ($P < 0.04$).

Discussion

Despite the fact that AD is known to have a genetic background, studies to date have failed to identify the genetic defects responsible for its development.¹² In the present study, we found associations between AD and genes encoding Th2-derived cytokines. Although it has been shown that some polymorphisms occur

Table 3 Association between SNPs and development of AD.

	<i>IL-17A</i>				<i>IL-23R</i>			
	<i>n</i> (%)		OR (95% CI)	<i>P</i>	<i>n</i> (%)		OR (95% CI)	<i>P</i>
	Patients (<i>n</i> = 166)*	Controls (<i>n</i> = 153)†			Patients (<i>n</i> = 162)*	Controls (<i>n</i> = 148)†		
Genotype								
G/G	65 (0.39)	69 (0.45)	Reference data	0.46	151 (0.93)	138 (0.93)	Reference data	1.00
G/A	83 (0.50)	74 (0.48)	1.19 (0.75–1.90)	0.13	11 (0.07)	10 (0.07)	1.01 (0.41–2.44)	–
A/A	18 (0.11)	10 (0.07)	1.91 (0.82–4.44)		0 (0.00)	0 (0.00)	–	
Allele								
G	213 (0.64)	212 (0.69)	Reference data		313 (0.97)	286 (0.97)	Reference data	1.00
A	119 (0.36)	94 (0.31)	1.26 (0.91–1.75)	0.17	11 (0.03)	10 (0.03)	1.01 (0.42–2.40)	
Missing data	–	7 (NK)			4	12		

AD, atopic dermatitis; NK, not known; SNP, single nucleotide polymorphism. Both groups were in Hardy–Weinberg equilibrium.

* $\chi^2 = 0.20$; † $\chi^2 = 1.81$.

Genotype: IL-17A–IL-23R	<i>n</i> (%)		OR (95% CI)	<i>P</i>
	Patients (<i>n</i> = 162)*	Controls (<i>n</i> = 144)		
G/G–G/G	62 (0.38)	63 (0.44)	Reference data	
G/G–G/A	2 (0.01)	2 (0.01)	1.02 (0.14–7.44)	0.69
G/G–A/A	0 (0.00)	0 (0.00)	–	–
G/A–G/G	77 (0.48)	61 (0.42)	1.28 (0.79–2.09)	0.32
G/A–G/A	4 (0.02)	8 (0.06)	0.51 (0.15–1.77)	0.28
G/A–A/A	0 (0.00)	0 (0.00)	–	–
A/A–G/G	12 (0.07)	10 (0.07)	1.22 (0.49–3.03)	0.67
A/A–G/A	5 (0.03)	0 (0.00)	–	–
A/A–A/A	0 (0.00)	0 (0.00)	–	–

* $P < 0.05$ statistically significant.

Table 4 *IL-17A/IL-23R* gene–gene interactions.

more frequently in patients with AD, the effect of these polymorphisms on the clinical course of the disease has not been established.^{12,13} In our previous study, we showed a link between the occurrence of the 2282del4 *FLG* mutation and the severity of AD, a higher risk of AD development, early onset of asthma and palmar hyperlinearity. We also showed significant correlations between a higher risk of AD development and the presence of the 2282del4 mutation and of IL-13 and IL-10 gene polymorphisms.¹⁴ Based on the data available in the literature and our own research results, we investigated the roles and interactions between genetic, immunological and environmental factors in AD pathogenesis. In the present study, we focused on the analysis of the association between two novel gene candidates predisposing to AD: IL-17 and IL-23. IL-17 is involved in innate immunity and IL-23 is involved in adaptive immunity, and both of these genes are now considered important pathogenic factors in inflammatory skin diseases, including AD.

IL-17 is a key cytokine in host defence in infections, as well as in the pathogenesis of autoimmune diseases.¹⁵ Many studies emphasize the fact that the number of Th17 cells circulating in the peripheral blood of patients with AD correlates with disease severity. These cells infiltrate the papillary layer of the dermis in AD lesions, especially in the acute phase of AD. Conversely, during the chronic phase of AD, the number of these cells is almost zero, and this phenomenon correlates with the reduced expression of AMPs and a higher risk of cutaneous superinfection.^{16,17} The fact that AD lesions are colonized chronically with *Staphylococcus aureus* confirms that Th1 and Th17 cells induce an innate immune response in the skin that is partially antagonized by Th2 cells. Furthermore, the lower flaggrin expression in AD lesions is consistent with the finding that Th2 cytokines inhibit expression of the flaggrin gene *in vitro*.¹⁸

The available data on the link between *IL-17A* SNPs and AD are limited. Ma *et al.*¹⁹ found that the A allele

Table 5 Association between *IL-17A* SNP and AD severity or coexistence of AD and asthma.

Genotype	n (%)		OR (95% CI)	P	n (%)		OR (95% CI)	P
	Patients* (n = 83)	Controls (n = 153)			Patients (n = 50)	Controls (n = 153)		
G/G	28 (0.34)	69 (0.45)	Ref.	–	18 (0.36)	69 (0.45)	Ref.	–
G/A	43 (0.52)	74 (0.48)	1.41 (0.79–2.52)	0.24	24 (0.48)	74 (0.48)	1.24 (0.62–2.49)	0.30
A/A	12 (0.14)	10 (0.07)	2.96 (1.145–7.63)	0.02	8 (0.16)	10 (0.07)	3.07 (1.06–8.89)	0.04

*Severe + moderate AD. AD, atopic dermatitis.

in the *IL-17A* G152A SNP is linked with an increased risk of susceptibility to AD, and correlates with higher *IL-17* expression. It was also reported that the percentage of Th17 cells, the *IL-17* mRNA level and the *IL-17* serum concentration were increased in patients with AD and correlated with the severity of AD.^{20,21} However, no association was found between AD susceptibility and the *IL-17F* gene polymorphism 7488T/C, which was, to an extent, consistent with our results. Our study revealed no relationship between *IL-17A* gene polymorphisms and AD risk; however, a significant correlation between the presence of the A/A genotype in the –152 G/A *IL-17A* SNP and AD (and its severity) suggests that *IL-17* participates in the pathogenesis of AD and may influence its clinical course.

The decision to investigate *IL-23R* SNPs in AD in the present study was motivated by a large body of research that provides evidence for the significant involvement of the *IL-23/Th17* axis in inflammatory diseases.^{22,23} Genetic studies have shown that the less common A allele of the *IL-23R* SNP Arg381Gln provides protection against the development of autoimmune diseases.²⁴

Di Meglio *et al.*²⁵ investigated the functional foundation of a human disease-associated gene variant, and demonstrated that *IL-23R* Arg381Gln exerts its protective effects through selective attenuation of *IL-23*-induced Th17 cell effector function without interfering with Th17 differentiation. This highlights its importance in the protection against *IL-23*-induced tissue pathologies. Our research revealed no direct association between frequency of the SNP (rs11209026, Arg381Gln) and susceptibility to AD. Nevertheless, a positive outcome of the investigation of the functional effect of disease-associated SNPs suggests that a longitudinal group study is necessary to improve the statistical power of genetic association in the pathogenesis of AD, especially as the genetic variant that was the main focus in the present study is particularly rare in individuals of western European descent (1% AA homozygous).

Conclusions

The investigated SNPs in the *IL-17A* and *IL-23R* genes were not associated with susceptibility to AD in the study population. In the case of *IL-17A* polymorphisms, there was a trend towards a higher frequency of the A/A and G/A genotypes and the A allele in patients with AD. The occurrence of the A/A genotype in *IL-17A* predisposes to more severe AD and its coexistence with asthma. Further studies with independent samples are needed to confirm our results. Nevertheless, we estimated that the minimal sample size achieved 80% statistical power, and we thus believe that our findings are valid. Continuing research into the *IL-23/Th17* pathway should provide a better understanding of disease mechanisms in AD, which in turn could result in new therapeutic options.

What's already known about this topic?

- Multiple genes are involved in the genetic background of AD, and their roles vary in different populations.
- Studies to date have failed to identify the genetic defects that are indisputably responsible for AD development.

What does this study add?

- We aimed to investigate the association between *IL-17A* and *IL-23R* gene polymorphisms and AD development.
- There was no statistical significance between the examined *IL17A* and *IL-23R* polymorphisms and AD; however, the genotype A/A in *IL-17A* polymorphism predisposed to increased severity of AD and coexistence with asthma.

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