

SHORT REPORT

An association of *TLR2* –16934A>T polymorphism and severity/phenotype of atopic dermatitis

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Abstract

Background Toll-like receptor 2 gene (*TLR2*) –16934A>T polymorphism has been shown to be associated with severity of atopic dermatitis (AD) as measured using severity scoring of atopic dermatitis (SCORAD) index. Moreover, *TLR2* –16934A>T polymorphism has been associated with atopy and allergic disorders in farmers' children.

Objective The aim of this study was to evaluate an association between *TLR2* –16934A>T polymorphism and AD phenotype, including disease severity and concomitant atopic diseases, or potential serum markers of AD severity and also to find a molecular background of the clinical associations.

Methods Genotyping for *TLR2* –16934A>T polymorphism was performed in 130 consecutive adult ambulatory patients with AD. Total serum (TS) IgE levels, serum tryptase, plasma interleukin-6 and C-reactive protein were measured. In addition, luciferase assay and electrophoretic-mobility shift assay were conducted to assess the effect of –16934A>T polymorphism on transcriptional activity.

Results There was an inverse association of *TLR2* –16934TT genotype and/or –16934T allele with SCORAD, but not with TS IgE, tryptase or inflammatory markers. Interestingly, –16934AA genotype and/or –16934A allele were overrepresented in AD patients with concomitant asthma or a family history of atopy. In a subgroup analysis, *TLR2* –16934A>T polymorphism was associated with SCORAD, asthma, allergic conjunctivitis or family history of atopy in AD patients with TS IgE ≥106 IU/mL but not in those having TS IgE <106 IU/mL. Functional analyses showed that *TLR2* –16934T allele is associated with higher luciferase activity in human monocytic THP-1 cells and preferential binding of the THP-1-derived nuclear protein.

Conclusion *TLR2* –16934A>T polymorphism could be a genetic predictor of AD severity, the coexistence of asthma or atopic conjunctivitis as well as a family history of atopic diseases, especially in subjects having higher TS IgE. *TLR2* –16934A>T polymorphism affects transcriptional activity, which may at least in part account for the clinical associations observed for the –16934A>T polymorphism.

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Keywords

atopic dermatitis, IgE, polymorphism, SCORAD, *TLR2*, tryptase

Conflict of interest

None declared.

Introduction

Severity scoring of atopic dermatitis index is a clinical scoring system that is widely used to assess the severity of atopic dermatitis (AD).^{1–5} Recently, Oh *et al.*³ have identified a frequent and functionally relevant toll-like receptor (TLR) 2 (*TLR2*) gene (*TLR2*) –16934A>T (rs4696480)* polymorphism as a genetic marker of AD severity. They reported an increased representation of

*Polymorphic locus was identified using accession number according to the Single Nucleotide Polymorphism database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>) and Innate Immunity in Heart, Lung and Blood Disease Programs for Genomic Applications (http://www.pharmgat.org/IIPGA2/index_html).

the –16934AA genotype/–16934A allele among German AD patients with severe AD phenotype (SCORAD > 50) when compared with those with mild-to-moderate AD (SCORAD ≤ 50).³ In addition, Eder *et al.*⁶ reported on the association between (*TLR2*) –16934A>T polymorphism and asthma, current asthma symptoms, atopy or current hay fever symptoms in farmers' children. However, associations between this polymorphism and concomitant atopic diseases have not been evaluated in AD patients. In addition, the immediate molecular mechanism underlying the clinical observations remained unknown.^{3,6}

Until now no blood marker is available to reflect AD clinical scoring.⁴ It is also unknown whether *TLR2* –16934A>T (rs4696480) polymorphism may affect proteins potentially associated with AD severity or those known as circulating markers of enhanced inflammatory response, namely tryptase, total serum (TS) IgE, IL-6 or C-reactive protein (CRP).^{3,4,7,8}

Materials and methods

A total of 130 consecutive ambulatory Polish patients diagnosed with AD based on the commonly accepted criteria⁹ were recruited (Table 1, Table S1). Allergic asthma was diagnosed based on typical symptoms and spirometry criteria based on GINA 2008 guidelines (<http://www.ginasthma.org>). The diagnosis of allergic rhinitis

and food allergy was established based on medical history, physical examination, total serum IgE levels and positive intracutaneous skin tests.

Serum tryptase was measured using a fluoroenzyme immunoassay (Pharmacia, Uppsala, Sweden), serum IL-6 by an ELISA (R&D Systems, Abingdon, UK), TS IgE and high sensitivity CRP nephelometrically (Dade Behring, Marburg, Germany; normal value <3.0 mg/L).

Genotyping of *TLR2* –16934A>T polymorphism was conducted by restrictive digestion (*Hpy*188III, New England Biolabs, Ipswich, MA, USA) of the product obtained using –16934A>T amplification primers as described by Oh *et al.*³

Electrophoretic-mobility shift assay (EMSA) and reporter assay were conducted as described previously.¹⁰

Results

In the whole group of AD patients ($n = 130$), *TLR2* –16934A>T polymorphism demonstrated an association with SCORAD but not with the candidate blood markers of AD severity (Table 2). *TLR2* –16934AA homozygotes had higher SCORAD values than those having –16934TT genotype/–16934T allele. In subjects with TS IgE ≥ 106 IU/mL ($n = 62$), the association of *TLR2* –16934A>T polymorphism with SCORAD remained significant and the between-genotype differences in SCORAD values became even larger (Table 3). In this subgroup, no associations were observed between the candidate AD severity blood markers and *TLR2* –16934A>T polymorphism (Table 3). Neither SCORAD nor the candidate blood markers showed any association with *TLR2* –16934A>T polymorphism in the subgroup of AD patients with TS IgE <106 IU/mL (Table S2).

In the whole group of AD subjects ($n = 130$), *TLR2* –16934AA genotype and/or –16934A allele were associated with concomitant allergic asthma as well as a family history of atopy in AD patients but not with allergic rhinitis, allergic conjunctivitis or food allergy (Table 4). Those associations remained significant in the subgroup of AD patients with TS IgE ≥ 106 IU/mL ($n = 62$), in whom allergic conjunctivitis was also shown to be significantly associated with *TLR2* –16934A>T (Table 5). No such associations were observed in subjects having TS IgE <106 IU/mL only (Table S3).

In search for potential mechanisms underlying the associations described above, functional studies were conducted to assess the effect of the *TLR2* –16934A>T polymorphism on transcriptional activity. Luciferase assay conducted with human monocytic THP-1 cells demonstrated by 28% higher luciferase activity of –16934T-based constructs when compared with those carrying the –16934A allele in our model (Fig. 1a), suggesting that the *TLR2* –16934T allele is characterized by higher transcriptional activity. Furthermore, EMSA with a competitive assay demonstrated preferential binding of some THP-1-derived nuclear protein to the –16934T probe (Fig. 1b). We were not, however, able to predict any candidate transcription factor corresponding to the observed mobility pattern.

Table 1 Characteristics of the study group

	Atopic dermatitis patients ($n = 130$)	
	N	%
Gender		
Male	45	34.6
Female	85	65.4
Years from onset of the disease		
<1	3	2.3
≥1–<5	17	13.1
≥5–<10	13	10.0
≥10	97	74.6
Asthma	36	26.7
Allergic rhinitis	66	50.8
Allergic conjunctivitis	43	33.1
Food allergy	49	37.7
Family history of atopy*	55	42.3
	Mean ± SEM or median [IQR]	
Age, years	29.6 ± 0.98	
SCORAD	32.4 ± 1.3	
Total serum IgE, IU/mL	96.0 [225.0]	
CRP, mg/L	1.78 [1.42]	
Tryptase, µg/L	2.44 [0.90]	
IL-6, pg/mL	2.86 [1.00]	

SCORAD, severity scoring of atopic dermatitis index; IgE, Immunoglobulin E; CRP, C-reactive protein; IL-6, interleukin-6.

*Positive if present in at least one of the following persons: parents, siblings or grandparents.

Table 2 Quantitative parameters in atopic dermatitis patients ($n = 130$) with respect to toll-like receptor-2 gene -16934 A>T polymorphism

Quantitative parameter*	Genotypes†			P-values for comparisons between genotypes‡		
	AA ($n = 33$)	AT ($n = 73$)	TT ($n = 24$)	AA vs. TT	AA vs. AT+TT	AA+AT vs. TT
SCORAD§¶	37.7 $\pm$ 3.1	31.6 $\pm$ 1.6	27.4 $\pm$ 2.2	0.01	0.04	0.07
Tryptase, $\mu\text{g/L}$	2.34 [0.74]	2.43 [0.91]	2.54 [0.73]	0.38	0.58	0.43
IL-6, pg/mL	2.91 [1.40]	2.82 [0.96]	2.87 [0.75]	0.77	0.70	0.99
Total serum IgE, IU/mL	90.0 [196.0]	89.4 [272.5]	102.5 [194.0]	0.42	0.59	0.38
CRP, mg/L	1.78 [1.20]	1.78 [1.51]	1.85 [1.47]	0.95	0.81	0.96

SCORAD, severity scoring of atopic dermatitis index; IL-6, interleukin-6; IgE, Immunoglobulin E; CRP, C-reactive protein.

*Data presented as mean $\pm$ SEM or median [IQR], depending on the normality of the distribution as analysed by Lilliefors test.

†Genotypes distributed according to Hardy–Weinberg equilibrium ($P = 0.58$, χ^2 test).

‡Comparisons by unpaired Student's t -test or Mann–Whitney U -test, depending on the normality of the distribution.

§Significant independent predictors in a multiple linear regression model with the SCORAD as a dependent variable and, age, gender, total serum (TS) IgE levels (approximated to normality by square root transformation), presence of asthma or allergic conjunctivitis and TLR2 -16934 A>T polymorphism [additive (order: AA, AT, TT), dominant (order: AA, AT+TT) or recessive model (order: AA+AT, TT)] as independent variables: additive model, -16934A>T polymorphism [β (SE): -0.22 (0.09), $P = 0.01$] and TS IgE [β (SE): 0.23 (0.09), $P = 0.01$]; dominant model, -16934A>T polymorphism [β (SE): -0.19 (0.09), $P = 0.03$] and TS IgE [β (SE): 0.23 (0.09), $P = 0.01$]; recessive model, TS IgE [β (SE): 0.23 (0.09), $P = 0.01$].

¶Indirect comparisons for -16934A>T polymorphism distribution between AD subjects with SCORAD ≤ 50 (23 AA, 66 AT and 24 TT) and those with SCORAD > 50 (10 AA, 7 AT and 0 TT): genotype model ($P = 0.001$, χ^2 test), dominant model ($P = 0.002$, two-tailed Fisher's exact test), recessive model ($P = 0.04$, two-tailed Fisher's exact test) and allelic model ($P = 0.001$, two-tailed Fisher's exact test).

Table 3 Quantitative parameters in atopic dermatitis patients having total serum IgE levels of ≥ 106 IU/mL ($n = 62$) with respect to toll-like receptor-2 gene -16934 A>T polymorphism

Quantitative parameter	Genotypes			P-values for comparisons between genotypes		
	AA ($n = 16$)	AT ($n = 35$)	TT ($n = 11$)	AA vs. TT	AA vs. AT+TT	AA+AT vs. TT
SCORAD†‡	42.6 $\pm$ 4.9	33.5 $\pm$ 2.3	27.3 $\pm$ 2.7	0.02	0.02	0.07
Tryptase, $\mu\text{g/L}$ ‡	2.69 [1.16]	2.67 [1.60]	2.88 [1.11]	0.51	0.97	0.44
IL-6, pg/mL‡	2.98 [1.49]	3.02 [0.86]	3.00 [0.94]	0.90	0.81	0.68
Total serum IgE, IU/mL‡	292.5 [454.0]	367.0 [561.0]	230.0 [166.0]	0.45	0.85	0.29
CRP, mg/L‡	1.97 [1.26]	1.98 [1.33]	1.84 [1.25]	0.55	0.57	0.72

SCORAD, severity scoring of atopic dermatitis index; IL-6, interleukin-6; IgE, Immunoglobulin E; CRP, C-reactive protein.

*Data presented as mean $\pm$ SEM and comparisons made by unpaired Student's t -test.

†Significant independent predictors in a multiple linear regression model with the SCORAD as a dependent variable and, age, gender, total serum IgE levels (approximated to normality by square root transformation), presence of asthma or allergic conjunctivitis and TLR2 -16934 A>T polymorphism [additive (order: AA, AT, TT), dominant (order: AA, AT+TT) or recessive model (order: AA+AT, TT)] as independent variables: additive model, -16934A>T polymorphism [β (SE): -0.32 (0.14), $P = 0.03$]; dominant model, none; recessive model, none.

Analysis of several candidate blood markers in association with AD severity demonstrated that TS IgE alone correlated with SCORAD (Table 6).

Discussion

TLR2 genetic variants were extensively studied in the context of atopy and allergic disorders,^{3,6,11–15} including AD.^{3,12–14} One of them, a functionally relevant¹² but rather infrequent,^{3,13,14} R753Q (rs5743708) polymorphism was associated with the severe phenotype of AD,¹³ which was subsequently not replicated in two larger studies.^{3,14} However, very recently, Oh *et al.*³ have demonstrated an association between another TLR2 variant, a very common -16934A>T (rs4696480) polymorphism, and a severity of AD. In an AD patient group of similar size, we corroborated the

relationship between TLR2 -16934A>T polymorphism and SCORAD reported by Oh *et al.*³ We observed an association between -16934TT genotype/-16934T allele and lower AD severity in direct comparisons of SCORAD values between genotypes and, similarly to Oh *et al.*,³ in comparison of the genotypic and/or allelic proportions between the groups with mild/moderate and severe AD. The discrepancies between the results obtained in adults, previously by Oh *et al.*³ and currently by us, and those obtained in parent-offspring trios by Weidinger *et al.*,¹⁴ who in contrast found no relationship between -16934A>T (rs4696480) polymorphisms and AD severity, might result from different study designs. In addition, it may also be speculated that -16934A>T polymorphism could be a predisposing factor for no disease remission in adulthood.

Table 4 Clinical characteristics of atopic dermatitis patients ($n = 130$) with respect to toll-like receptor-2 gene (*TLR2*) –16934 A>T polymorphism

Clinical phenotype/feature	Genotype			T allele frequency	P-values			
	AA ($n = 33$), N (%)	AT ($n = 73$), N (%)	TT ($n = 23$), N (%)		AA vs. AT vs. TT*	AA vs. AT+TT†	AA+AT vs. TT†	A vs. T†
Asthma‡								
–	18 (19.1)	54 (57.4)	22 (23.4)	0.521	0.007	0.01	0.02	0.004
+	15 (41.7)	19 (52.8)	2 (5.6)	0.319				
Allergic rhinitis								
–	16 (25.0)	36 (56.2)	12 (18.7)	0.469	0.99	1.0	1.0	1.0
+	17 (25.8)	37 (56.1)	12 (18.2)	0.462				
Allergic conjunctivitis								
–	20 (23.0)	47 (54.0)	20 (23.0)	0.500	0.16	0.40	0.09	0.12
+	13 (30.2)	26 (60.5)	4 (9.3)	0.395				
Food allergy								
–	24 (29.6)	40 (49.4)	17 (21.0)	0.457	0.13	0.21	0.48	0.80
+	9 (18.4)	33 (67.3)	7 (14.3)	0.480				
Family history of atopy§								
–	11 (14.7)	46 (61.3)	18 (24.0)	0.547	0.003	0.002	0.07	0.002
+	22 (40.0)	27 (49.1)	6 (10.9)	0.354				

*By chi-square test.

†By two-tailed Fisher's exact test.

‡Significant independent predictors in a multiple linear regression model with the presence of asthma (order: absence, presence) as a dependent variable and, age, gender, total serum (TS) IgE levels (approximated to normality by square root transformation), and *TLR2* –16934 A>T polymorphism [additive (order: AA, AT, TT), dominant (order: AA, AT+TT) or recessive model (order: AA+AT, TT)] as independent variables: additive model, –16934A>T polymorphism [β (SE): –0.24 (0.08), $P = 0.004$] and TS IgE [β (SE): 0.24 (0.08), $P = 0.004$]; dominant model, –16934A>T polymorphism [β (SE): –0.20 (0.08), $P = 0.02$] and TS IgE [β (SE): 0.25 (0.08), $P = 0.004$]; recessive model, –16934A>T polymorphism [β (SE): –0.18 (0.08), $P = 0.03$] and TS IgE [β (SE): 0.26 (0.08), $P = 0.003$].§Significant independent predictors in a similar model as described above (‡), in which asthma was replaced with family history of atopy (order: negative, positive): additive model, –16934A>T polymorphism [β (SE): –0.27 (0.08), $P = 0.002$] and TS IgE [β (SE): 0.17 (0.09), $P < 0.05$]; dominant model, –16934A>T polymorphism [β (SE): –0.28 (0.08), $P = 0.001$] and TS IgE [β (SE): 0.17 (0.09), $P = 0.04$]; recessive model, TS IgE [β (SE): 0.19 (0.09), $P = 0.03$].

Of note, our study extended the previous observations by showing that *TLR2* –16934A>T polymorphism was associated with concomitant asthma or family history of atopy in AD patients. Eder *et al.*⁶ showed that farmers' children carrying the –16934T allele were less likely to have a diagnosis of asthma, current asthma symptoms, atopy and current hay fever symptoms. Interestingly, we found that the –16934AA genotype/–16934A allele (rs4696480) is overrepresented among those AD patients who have concurrent asthma or family history of atopy. It suggests that a genetic link between –16934A>T polymorphism and asthma or atopy described by Eder *et al.*⁶ exists also in AD patients or their families, although another study by Smit *et al.*¹⁵ failed to show any association between *TLR2* –16934A>T polymorphism and asthma or atopy in young adult farmers.

Two clinical forms of AD have been identified based on the presence (extrinsic form) or absence (intrinsic form) of IgE-mediated sensitization.^{2,16} A cut-off value of 106 IU/mL for TS IgE levels has been reported to be a reliable and useful parameter to differentiate between intrinsic and extrinsic AD in children.¹⁶ To further extend our observations, we divided our patients into two groups based on TS IgE level cut-off value of 106 IU/mL.¹⁶

Of interest, the associations between *TLR2* –16934A>T polymorphism and SCORAD, asthma and family history of atopy observed in our whole group of AD patients ($n = 130$) were also present and even more pronounced in the subgroup of patients with TS IgE levels ≥ 106 IU/mL. In addition, *TLR2* –16934A>T polymorphism was significantly associated with allergic conjunctivitis in this subgroup. On the contrary, no genotype-related associations were observed in the subgroup with TS IgE levels < 106 IU/mL. Together, it might suggest that the effects observed for *TLR2* –16934A>T polymorphism in AD subjects are restricted to those having the extrinsic form of the disease as defined by TS IgE levels ≥ 106 IU/mL, which may be in line with the hypothetical underlying mechanism that supports the development of atopy (see further).

Phenotypic differences associated with the genotypes of –16934A>T (rs4696480) polymorphism were found in this study as well as have been reported by the other investigators.^{3,6} The observation of markedly higher IL-6 production by Pam₃-Cys-stimulated PBMCs¹⁷ or whole blood³ obtained from donors with –16934AA genotype when compared with those obtained from those carrying the –16934T allele¹⁷ or having –16934TT genotype³

Table 5 Clinical characteristics of atopic dermatitis patients having total serum IgE levels of ≥ 106 IU/mL ($n = 62$) with respect to toll-like receptor-2 gene (*TLR2*) -16934 A>T polymorphism

Clinical phenotype/feature	Genotype			T allele frequency	P-values			
	AA ($n = 16$), n (%)	AT ($n = 35$), N (%)	TT ($n = 11$), N (%)		AA vs. AT vs. TT*	AA vs. AT+TT†	AA+AT vs. TT†	A vs. T†
Asthma‡								
–	4 (10.5)	24 (63.2)	10 (26.3)	0.579	0.001	0.001	0.04	0.0009
+	12 (50.0)	11 (45.8)	1 (4.2)	0.271				
Allergic rhinitis								
–	6 (27.3)	11 (50.0)	5 (22.7)	0.477	0.68	1.0	0.50	0.85
+	10 (25.0)	24 (60.0)	6 (15.0)	0.450				
Allergic conjunctivitis§								
–	7 (18.4)	20 (52.6)	11 (28.9)	0.553	0.01	0.14	0.004	0.01
+	9 (37.5)	15 (62.5)	0 (0.0)	0.312				
Food allergy								
–	12 (31.6)	18 (47.4)	8 (21.0)	0.447	0.19	0.24	0.51	0.85
+	4 (16.7)	17 (70.8)	3 (12.5)	0.479				
Family history of atopy¶								
–	4 (12.1)	21 (63.6)	8 (24.2)	0.561	0.02	0.02	0.19	0.02
+	12 (41.4)	14 (48.3)	3 (10.3)	0.344				

*By chi-square test.

†By two-tailed Fisher's exact test.

‡Significant independent predictors in a multiple linear regression model with the presence of asthma (order: absence, presence) as a dependent variable and, age, gender, total serum (TS) IgE levels (approximated to normality by square root transformation) and *TLR2* -16934 A>T polymorphism [additive (order: AA, AT, TT), dominant (order: AA, AT+TT) or recessive model (order: AA+AT, TT)] as independent variables: additive model, -16934A>T polymorphism [β (SE): -0.42 (0.12), $P = 0.0006$]; dominant model, -16934A>T polymorphism [β (SE): -0.41 (0.12), $P = 0.0009$]; recessive model, -16934A>T polymorphism [β (SE): -0.26 (0.13), $P < 0.05$].§Significant independent predictors in a similar model as described above (‡), in which asthma was replaced with allergic conjunctivitis (order: negative, positive): additive model, -16934A>T polymorphism [β (SE): -0.32 (0.12), $P = 0.01$]; dominant model, none; recessive model, -16934A>T polymorphism [β (SE): -0.34 (0.12), $P = 0.007$].¶Significant independent predictors in a similar model as described above (‡), in which asthma was replaced with family history of atopy (order: negative, positive): additive model, -16934A>T polymorphism [β (SE): -0.30 (0.12), $P = 0.02$]; dominant model, -16934A>T polymorphism [β (SE): -0.29 (0.12), $P = 0.02$]; recessive model, TS IgE [β (SE): 0.28 (0.13), $P = 0.04$].

has been suggested to explain to some extent the phenotypic differences.³ The differences in IL-6 production observed upon the Pam₃-Cys-stimulation^{3,17} were not present in resting cells,³ which may probably explain the lack of -16934A>T polymorphism-related differences in serum IL-6 levels in AD patients. In addition, local IL-6 effects, which are not reflected by its serum levels, cannot be excluded.

The immediate molecular mechanism underlying the relationships between -16934A>T polymorphism and atopy remained unknown.^{3,6} Lower expression/activity of TLR2 might be expected to result in decreased Th1 response and thus more pronounced Th2 responses, leading to atopy.¹¹ Carriers of the -16934T allele were speculated to have increased expression of TLR2 on the surface of antigen-presenting cells because of putative regulatory molecular effects of -16934A>T polymorphism, resulting in a reduced risk of asthma or allergic diseases.⁶ Indeed, we observed that reporter constructs carrying the -16934T allele exhibited significantly higher transcriptional activity than those having the -16934A allele, although the effect observed in our model may be of limited biological relevance because of its limited magnitude

and the differences between experimental conditions and those present *in vivo*. Furthermore, -16934T allele demonstrated preferential binding of nuclear protein possibly corresponding to some unknown activating transcription factor. One cannot, however, exclude that some other, yet unidentified polymorphisms in linkage disequilibrium with -16934A>T may also contribute to the observed phenotypic effects.^{3,6} It should also be highlighted that no -16934A>T-related differences have been observed in constitutive *TLR2* mRNA levels³ measured in monocytes obtained from healthy subjects. Nevertheless, it would be interesting to analyse the *TLR2* expression in monocytes obtained from the AD patients to find a missing link between the clinical findings^{3,6} and the molecular data reported here. Such an analysis of expression was, however, beyond the scope of this study.

We also tested several candidate serum markers of AD severity as measured by SCORAD, including TS IgE, IL-6, CRP and trypsinase. We observed a correlation between TS IgE levels and the severity of AD, which is in contrast to the study by Gerdes *et al.*,⁴ who reported that TS IgE levels did not correlate with AD severity as measured using SCORAD. This inconsistency might, however,

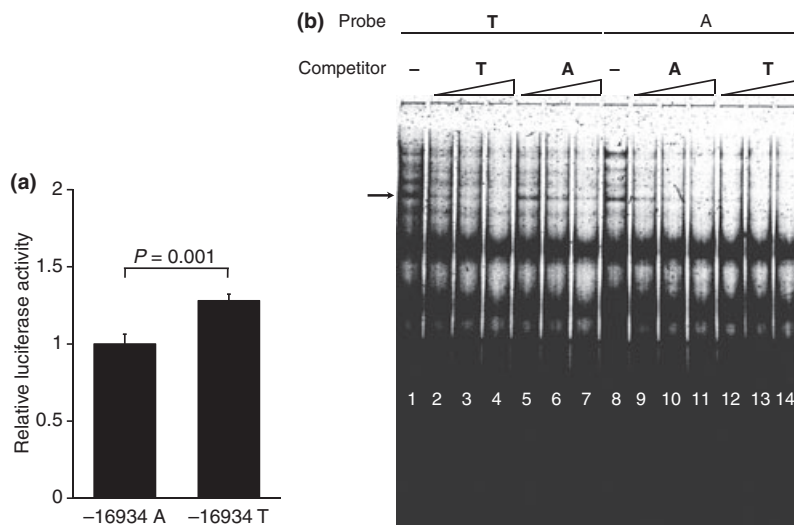


Figure 1 (a) Effect of toll-like receptor 2 gene (*TLR2*) –16934 A>T polymorphism on transcriptional activity. Triple, repeating motif of TGCATCTGG(A/T)GAGGGTCAT, covering for –16934 A>T polymorphism within its genomic neighbourhood, was *XhoI/HindIII*-subcloned upstream of the minimal promoter of pGL4.23[*luc2*/miniP] plasmid (Promega, Madison, WI, USA). Reporter assay was conducted in human monocytic THP-1 cells. Luciferase activity of each reporter plasmid was normalized against Renilla luciferase activity driven by pRL-CMV (used as the internal control) and is relative to that of the less active reporter plasmid (–16934 A). The results are mean + SEM for four independent experimental series conducted in triplicate. Comparison was made by unpaired Student's *t*-test. (b) Effect of *TLR2* –16934 A>T polymorphism on EMSA profile and preferential binding of nuclear protein to –16934 T-specific probe. EMSA was performed with FITC-labelled AAGGGCTGCATCTGG(A/T)GAGGGTCATCTGGCT double-stranded probes carrying –16934 T allele (lane 1–7) or –16934 A allele (lane 8–14) and nuclear extracts from THP-1 cells. For competition assay, 25-, 50- and 100-fold levels of –16934 T (lanes 2–4 and 12–14) or –16934 A (lanes 5–7 and 9–11) unlabelled double-stranded competitors of the sequence identical with the corresponding probes were used. Specific band, which distinguishes the difference of –16934 A>T polymorphism, is indicated with an arrowhead.

Table 6 Correlations between the severity of atopic dermatitis (AD) as measured by severity scoring of AD index (SCORAD) and its candidate blood markers in whole group of AD patients ($n = 130$) and its subgroups determined by a cut-off value of total serum IgE levels of 106 IU/mL (<106 IU/mL, $n = 68$; ≥ 106 IU/mL, $n = 62$)

	AD patients ($n = 130$)	
	R^*	P -values*
Tryptase	0.05	0.57
IL-6	0.10	0.26
Total serum IgE	0.23	0.01†
CRP	0.11	0.24

IgE, Immunoglobulin E; IL-6, interleukin-6; CRP, C-reactive protein.

*Correlations calculated by Spearman's Rank Correlation Coefficient.

†Significant independent predictors in a multiple linear regression model with SCORAD as a dependent variable and, tryptase, IL-6, total serum (TS) IgE levels, CRP (all four variables approximated to normality by square root transformation), age and gender as independent variables, in the whole group of AD patients ($n = 130$): TS IgE [β (SE): 0.25 (0.11), $P = 0.03$].

result from the fact that Gerdes *et al.*⁴ studied consecutive hospitalized AD patients on admission and at discharge, while we enrolled a larger group of consecutive ambulatory patients. On the

other hand, the two studies were consistent regarding lack of a correlation between serum tryptase and SCORAD.

IL-6 production by peripheral blood mononuclear cells (PBMCs) was reported to inversely correlate with sleep efficiency, which in turn negatively correlated with AD severity in a smaller population with mild-to-moderate disease.⁸ However, we found no correlation between plasma IL-6 and AD severity, although, again, local IL-6 effects may not be excluded.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Characteristics of the study participants with respect to total serum IgE levels (cutoff value of 106 IU/mL).

Table S2 Quantitative parameters in atopic dermatitis patients having total serum IgE levels of <106 IU/mL (*n* = 68) with respect to toll-like receptor-2 gene -16934 A>T polymorphism.

Table S3 Clinical characteristics of atopic dermatitis patients having total serum IgE levels of <106 IU/mL (*n* = 68) with respect to toll-like receptor-2 gene (TLR2) -16934 A>T polymorphism.

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Supplementary table 1 Characteristics of the study participants with respect to total serum IgE levels (cutoff value of 106 IU/mL)

		Total serum IgE levels <106 IU/mL (n=68)		Total serum IgE levels ≥106 IU/mL (n=62)	
		N	%	N	%
Sex					
	Male	24	35.3	21	33.9
	Female	44	64.7	41	66.1
Years from onset of the disease					
	< 1	1	1.5	2	3.2
	≥1 – <5	11	16.2	6	9.7
	≥5 – <10	4	5.9	9	14.5
	≥10	52	76.5	45	72.6
Asthma		12	17.6	24	38.7
Allergic rhinitis		26	38.2	40	64.5
Allergic conjunctivitis		19	27.9	24	38.7
Food allergy		25	36.8	24	38.7
Family history of atopy*		26	38.2	29	46.8
		Mean ± SEM or median [IQR]		Mean ± SEM or median [IQR]	
Age, years		30.4 ± 1.4		28.8 ± 1.3	
SCORAD		30.2 ± 1.7		34.7 ± 1.9	
Total serum IgE, IU/mL		65.2 [44.7]		290.0 [499.0]	

CRP, mg/L	1.63 [1.38]	1.93 [1.26]
Tryptase, µg/L	2.28 [0.64]	2.70 [1.27]
IL-6, pg/mL	2.74 [0.78]	3.01 [1.19]

SCORAD, severity scoring of atopic dermatitis index; IgE, Immunoglobulin E; CRP, C-reactive protein; IL-6, interleukin-6.

*Positive if present in at least one of the following persons: parents, siblings or grandparents.

Supplementary table 2 Quantitative parameters in atopic dermatitis patients having total serum IgE levels of <106 IU/mL (n=68) with respect to toll-like receptor-2 gene –16934 A>T polymorphism

Quantitative parameter	Genotypes			<i>P</i> -values for comparisons between genotypes		
	AA (n=17)	AT (n=38)	TT (n=13)	AA vs. TT	AA vs. AT+TT	AA+AT vs. TT
SCORAD*	33.0 ± 3.6	29.9 ± 2.4	27.6 ± 3.6	0.30	0.36	0.46
Tryptase, µg/L†	2.26 [0.40]	2.30 [0.65]	2.40 [0.51]	0.48	0.40	0.73
IL-6, pg/mL†	2.82 [1.11]	2.71 [0.71]	2.78 [0.58]	0.68	0.87	0.68
Total serum IgE, IU/mL†	69.0 [42.0]	65.6 [38.8]	38.2 [48.6]	0.32	0.31	0.35
CRP, mg/L†	1.33 [0.84]	1.70 [1.47]	1.76 [1.71]	0.82	0.49	0.96

SCORAD, severity scoring of atopic dermatitis index; IL-6, interleukin-6; IgE, Immunoglobulin E; CRP, C-reactive protein.

*Data presented as mean ± SEM and comparisons made by unpaired Student's t-test.

†Data presented as median [IQR] and comparisons made by Mann-Whitney U-test.

Supplementary table 3 Clinical characteristics of atopic dermatitis patients having total serum IgE levels of <106 IU/mL (n=68) with respect to toll-like receptor-2 gene (*TLR2*) –16934 A>T polymorphism

Clinical phenotype/feature		Genotype			T allele frequency	P-values			
		AA (n=17), n (%)	AT (n=38), N (%)	TT (n=13), N (%)		AA vs. AT vs. TT*	AA vs. AT+TT†	AA+AT vs. TT†	A vs. T†
Asthma									
	–	14 (25.0)	30 (53.6)	12 (21.4)	0.482	0.55	1.0	0.44	0.65
	+	3 (25.0)	8 (66.7)	1 (8.3)	0.412				
Allergic rhinitis									
	–	10 (23.8)	25 (59.5)	7 (16.7)	0.464	0.72	0.78	0.54	0.86
	+	7 (26.9)	13 (50.0)	6 (23.1)	0.481				
Allergic conjunctivitis									
	–	13 (26.5)	27 (55.1)	9 (18.4)	0.459	0.89	0.76	1.0	0.70
	+	4 (21.1)	11 (57.9)	4 (21.1)	0.500				
Food allergy									
	–	12 (27.9)	22 (51.2)	9 (20.9)	0.465	0.59	0.57	0.75	1.0
	+	5 (20.0)	16 (64.0)	4 (16.0)	0.480				
Family history of atopy									
	–	7 (16.7)	25 (59.5)	10 (23.8)	0.536	0.10	0.08	0.34	0.08
	+	10 (38.5)	13 (50.0)	3 (11.5)	0.365				

*By χ^2 test.

†By two-tailed Fisher's exact test.