Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 30 (3), 2023: 102–109.
www.Ejimmunology.org

NLRP3 inflammasome (rs10754558) gene polymorphism in patients with atopic dermatitis

Aya El Gendy[1], Fawzia H. Abo Ali[1], Shereen A. Baioumy[2], Sara I. Taha[3], Mahy El-Bassiouny[4], and Osama M. Abdel Latif[1]

[1]Department of Internal Medicine, Allergy & Clinical Immunology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

[2]Department of Microbiology & Immunology, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

[3]Department of Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

[4]Department of Dermatology, Andrology & Venereology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Corresponding author: Sara I. Taha, Department of Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.
Email: dr_sara_ib@med.asu.edu.eg.

Abstract

The nucleotide-binding oligomerization domain-like receptor 3 (NLRP3) inflammasome is a high molecular weight protein complex that has been linked to a variety of allergic and inflammatory disorders in humans, including atopic dermatitis (AD). Polymorphisms in NLRP3 genes could lead to immune dysregulation. This case-control study aimed to assess the association between NLRP3 inflammasome (rs10754558) gene polymorphism in AD and the incidence and severity of the disease. We included 62 subjects in each of the AD and control groups. Serum total IgE levels and NLRP3 inflammasome (rs10754558) gene polymorphism were assessed and compared between the two study groups and among the AD group as arranged by disease severity. The AD group showed significantly higher levels of serum total IgE compared to controls (p<0.001). Serum IgE levels were also significantly associated with AD severity. The (rs10754558) G allele was significantly predominant among AD participants (OR: 2.33; 95% CI: 1.1 -4.92) and 51.6% of the AD group was carriers of the GG genotype. Moreover**,** there was a substantial correlation between NLRP3 (rs10754558) G allele and AD score index for disease severity (OR: 7.17; 95% CI: 1.47 – 35.7). In conclusion, NLRP3 inflammasome (rs10754558) gene polymorphism G allele could be an important factor in the predisposition and exacerbation of AD.

Keywords: Allergy, Atopic dermatitis, Gene polymorphism, Inflammasome, NLRP3.

Date received: 21 January 2023; **accepted:** 07 June 2023

Introduction

Atopic dermatitis (AD) is an ongoing inflammation of the skin, characterized by dry skin, itching, lichenification in addition to recurrent eczematous scratches in response to allergens in a setting of abnormal immune response. The pathogenesis of AD is multifaceted with several collaborative factors influencing the disease.[1] The most important factors are genetic factors, epidermal barrier defect, an impaired immune response and

unbalanced microbiology of the skin which result in impaired protection against external environmental exacerbating factors. The pathophysiology of the disease involves both innate and adaptive immunological responses.[1]

Intracellular and extracellular pattern recognition receptors (PRRs) are crucial parts of innate immunity upon which their activation caspase 1-dependent inflammatory events occur. There are several PRRs families in humans including toll-like receptors (TLRs), nucleotide binding leucine-rich repeat-containing proteins/nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs).[2]

NOD-like receptor protein3 (NLRP3) inflammasome is a significant regulator in the immune system, its stimulation leads to different immunological diseases.[2] Stimulation of NLRP3 inflammasome through the interior environment, metabolic disturbance, and infective micro-organisms can induce T-helper (Th)-2 cytokines which are key players in AD pathogenesis.[3] Inhibitors of NLRP3 inflammasome may become a possible treatment option for different allergic diseases.[4] The interruption of NLRP3 inflammasomes may recover glucocorticoids treatment sensitivity in inflammatory skin diseases such as AD and psoriasis in mouse models.[5]

The messenger ribonucleic acid (mRNA) and expression levels of inflammatory mediators in the process of inflammation are affected by NLRP3 gene variants[6] which can affect the threshold of inflammasome stimulation and disrupt the coordinated immune reaction.[7] NLRP3 inflammasome rs10754558 SNPs was linked to the susceptibility to other autoimmune and inflammatory illness in several studies. Hitomi et al., 2009, reported that hypersensitivity conditions such as food-induced anaphylaxis and aspirin induced asthma are associated with NLRP3 (rs10754558) SNP.[8] It was also reported that the G allele of the rs10754558 SNP was associated with gouty arthritis,[9] and the severity of coronary artery stenosis.[10] In addition, Lee & Bae, 2016, reported that NLRP3 rs10754558 C/G SNP are linked to vulnerability to systemic lupus erythematosus.[11] Also, Pontillo et al., 2010, linked NLRP3 rs10754558 SNP to type 1 diabetes in the Brazilian population.[12]

The current case-control study aimed at investigating the relationship between the NLRP3 inflammasome (rs10754558) single nucleotide polymorphism (SNP) and AD disease incidence and severity.

Subjects and Methods

This case-control study included 62 adult individuals with AD (both males and females) aged ≥ 18 years and recruited from the Allergy and Clinical Immunology outpatient clinic at Zagazig University Hospitals and Ain Shams University Hospitals. In addition, 62 apparently healthy age-and gender-matched were included as control subjects. The diagnosis of AD followed the diagnostic criteria of the American Academy of Dermatology.[13] Patients having cancer, active infection, autoimmune or collagen diseases, physical urticaria, urticarial vasculitis or those on immunomodulating drugs or antihistamines for the previous 7 days were excluded from the study.

The included participants with AD were classified according to the SCORing Atopic Dermatitis (SCORAD) index;[7] severe index was recorded in 24 patients (37.7%); mild index in 22 patients (35.5%) and moderate index in 16 patients (25.8%).

All participants were subjected to detailed history taking and examination. A venous blood sample (4 ml) was obtained from each subject under complete aseptic conditions. Of these, an aliquot of 2 ml was delivered into a plain vacutainer tube, serum separated after centrifugation at 2500 ×g for fifteen minutes and used for assessment of total immunoglobulin (Ig)-E by ELISA. The other aliquot (2 ml) was delivered into an EDTA vacutainer tube for the polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP). All samples were stored at -80°C until used.

Serum total IgE level quantitation

IgE was assessed using a Human Total IgE ELISA commercial kit (IMMUNOSPEC Corporation, Canoga Park, CA, USA), according to the

manufacturer's instructions. The optical density was read at 450 nm with a microtiter plate reader (BioTek, Winooski, VT, USA). The minimum detectable concentration of IgE by the used kit was 5.0 IU/ml. Total IgE level in a normal, allergy-free adult is less than 150 IU/ml.

Determination of inflammasome NLRP3 (rs10754558) SNP

The NLRP3 (rs10754558) gene was amplified using the direct blood PCR commercial kit (Phusion™ Blood Direct PCR Master Mix; Thermo Scientific™, USA), according to the manufacturer's instructions. The reaction mixture consisted of 10 µL of 2× Phusion Blood Direct PCR Master Mix, one µL of whole blood without prior sample preparation or DNA extraction, 0.5 µM of each primer, 0.6 µL of MgCl2, and the volume was completed to a final volume 20 µL by RNase free water. The sequence of primers used for the human NLRP3 (rs10754558) gene was as follows:

Forward:
5'CAGGACAATGACAGCATCGGGTGTTGAT-3',
Reverse:
5-'GCTGCCATAAAATTTCAACATAA-3'

The PCR was performed using a thermal cycler (Veriti thermal cycler, Applied Biosystems, Singapore) with the following conditions: denaturation at 95◦C for 5 min, followed by 35 cycles each of denaturation at 95◦C for 30 s, annealing at 58.5 ◦C for 30 s, and extension at 72◦C for 30 s, followed by a final extension at 72◦C for 5 min. The PCR product size was 261 bp.

The post-amplification analysis was carried out by directly loading 5 µL of the O'GeneRuler DNA Ladder mix included in the kit and ten µL of the PCR mixture onto a 1.5% agarose gel ethidium-bromide stained for electrophoresis examination. PCR products were seen under ultraviolet light.

The 261 bp PCR product was digested with MboI restriction enzyme (Thermo Scientific™, USA) according to the manufacturer's protocol**.** For this, 10 µL of PCR reaction mixture, 7 µL nuclease-free water, two µL 10X green buffer and one µL MboI were inserted in 0.2 mL Eppendorf tube**.** The digested products were loaded on ethidium-bromide-stained agarose gel and electrophoresed then visualized under ultraviolet light. The NLRP3 rs10754558 genotypes were detected based on their size (Figure 1).

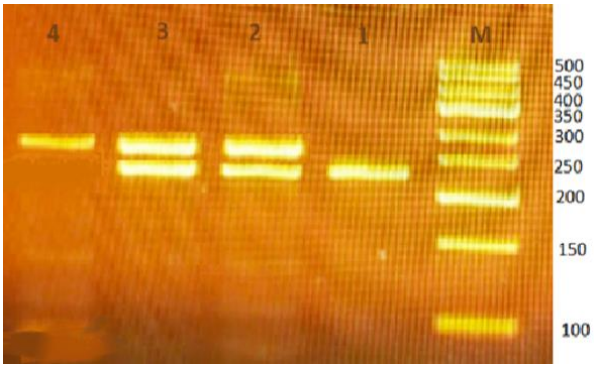

Figure 1. NLRP3 rs10754558 SNP by PCR-RFLP gel electrophoresis. Lane M: DNA Ladder (500 bp), Lane 1: CC genotype (236 bp), Lanes 2 & 3: GC genotype (261 bp & 236 bp), Lane 4: GG genotype (261 bp).

Statistical analysis

Data were analyzed by using the Statistical Package for the Social Sciences (SPSS)© software version 23. Numerical variable amount of continuous variant of normally dispersed statistics was specified as mean and standard deviation (SD). Independent-samples t-test was used to compare between 2 groups but one-way analysis of variance (ANOVA) was utilized for comparison between multiple-group. Categorical data was verified as number and percentage; variances were assessed by the chi-squared test and Fisher's exact test. Multiple logistic regression models were accomplished. A *p*-value < 0.05 was considered statistically significant. Odds ratios (ORs) were presented with 95% confidence intervals (95%CI).

Results

Table 1 illustrates the demographic data of AD participants and controls. AD was significantly associated with higher levels of serum total IgE compared to control subjects ($p<0.001$) (Table 2). According to the SCORAD index's assessment of the severity of AD, no discernible changes in serum total IgE levels were found. ($p=0.935$). The mean ±SD serum IgE level in the severe atopic dermatitis subgroup was 115.25±33.31

IU/ml while 122.55±37.41 IU/ml and 114.63±61.64 IU/ml in the mild and moderate subgroups, respectively. Serum total IgE levels did not differ significantly between the three genotypes of the inflammasome NLRP3 (rs10754558) (p=0.710). The mean ±SD serum IgE level in the GG genotype carriers was 124.55±38.15 IU/ml while 119.25±32.31 IU/ml and 115.63±61.34 IU/ml in the GC and CC genotype carriers, respectively.

Table 1. Comparison of the demographic characteristics between the 62 AD participants and 62 controls.

	AD group	Control group	p-value
Gender n., (%)			
Female	28 (45.2)	36 (58.1)	NS
Male	34 (54.8)	26 (41.9)	
Residence n, (%)			
Rural	38 (61.3)	38 (61.3)	NS
Urban	24 (38.7)	24 (38.7)	
Occupation n. (%)			
Employed	28 (45.2)	36 (58.1)	NS
Unemployed	34 (54.8)	26(41.9)	
Smoking n, (%)			
Yes	44 (71)	40 (64.5)	NS
No	18 (29)	22 (35.5)	
Age (years)			
Mean ± SD	34 ± 10.84	36.13 ± 11.59	NS
Range	17 - 56	18 - 58	

$P > 0.05$ is not significant (NS).

Table 2. Serum total IgE levels compared between the 62 AD participants and the 62 controls.

	AD group	Control group n.= 62	p-value
Total IgE (IU/ml)			
Mean±SD	117.68 ± 42.11	84.4 ± 18.6	<0.001
Range	20 - 240	65.8-103	

*$P \leq 0.05$ is significant.

The frequencies of the inflammasome NLRP3 (rs10754558) genotype and allele expression differed significantly between AD individuals and controls (p=0.044 and p=0.026, respectively). The (rs10754558) G allele was significantly predominant among AD group (OR: 2.33; 95% CI: 1.1 -4.92) and 51.6% of the AD group was carriers of the GG genotype (OR: 3.07; 95% CI: 1.05- 8.93), Table 3.

Table 3. Comparison between the studied groups according to the frequencies of NLRP3 inflammasome (rs10754558) genotypes and alleles.

NLRP3 inflammasome (rs10754558)		AD group n=62	Control group n=62	*p-value*	OR (95% CI)
Genotype n, (%)	GG	32 (51.6)	16 (25.8)	0.044	3.07 (1.05- 8.93)
	CG	26 (41.9)	34 (54.8)		
	CC	4 (6.5)	12 (19.4)		
Allele n, (%)	G	90 (72.5)	66 (53.2)	0.026	2.33 (1.1 -4.92)
	C	34 (27.5)	58 (46.8)		

*$P \leq 0.05$ is significant.

Additionally, there was a strong correlation between the G allele of NLRP3 (rs10754558) and the SCORAD score for the severity of AD (OR: 7.17; 95% CI: 1.47 – 35.7). GG genotype carriers had more severe AD with increased odds of severe lesion by 10.83 folds, Table 4.

Table 4. Frequencies of NLRP3 (rs10754558) genotypes and alleles according to AD severity by SCORAD index.

NLRP3 (rs10754558)		**Mild**	**Moderate**	**Severe**	***p-value***	**OR (95% CI)**
		n=22	n=16	n =24		
Genotype n, (%)	GG	10 (45.5)	12 (75)	20 (83.3)	0.021	10.83 (1.79 – 65.55)
	CG	8 (36.4)	4 (25)	4 (16.7)		
	CC	4 (18.2)	0 (0.0)	0 (0.0)		
Allele n, (%)	G	28 (63.6)	28 (87.5)	44 (91.7) 4 (8.3)	0.022	7.17 (1.47 – 35.7)
	C	16 (36.4)	4 (12.5)			

*$P \leq 0.05$ is significant.

Discussion

AD is a complex inflammatory skin condition caused by a confluence of various genetic and environmental variables. It is crucial to identify the genetic components of AD to develop innovative treatment and preventative methods.[14,15] NLRP3 is the most well-known inflammasome which has been linked to various inflammatory conditions.[16] The current study aimed at investigating the association between NLRP3 inflammasome gene (rs10754558) SNP and AD incidence and severity.

Male predominance (54.8%) was observed among our included AD participants. A finding that goes along with what was reported by previous studies.[17,18] As sex hormones frequently have an impact on the immune system.[19]

Most of our included AD participants were residents of rural areas (61.3%), unemployed (54.8%) and non-smokers (71.0%). Our findings are contrary to earlier studies, which revealed that AD was more common in urban than rural areas.[20,21] The discrepancy could be explained by age and gender difference between study populations as well as the availability of medical care and proper treatment.

Similar to our data, Andersen et al., 2020,[22] and Yano et al., 2013,[23] reported that AD decreased activity and work productivity and resulted in increased unemployment. Also, Kantor et al., 2016, reported that the prevalence of AD increased by active and passive exposure to smoking which probably has numerous possessions on the immune system and barrier function of the skin that might lead to AD.[24]

The current study revealed that serum IgE levels were significantly higher in AD patients than controls (p<0.001) but showed no significant association with AD severity by SCORAD index (p=0.935) nor NLRP3 inflammasome (rs10754558) genotypes. Sehgal et al., 2015, studied 100 newly diagnosed AD patients of various ages and found that mean serum IgE levels were 1084.73 IU/ml (± SD 776.27 IU/ml) with a range of 72-3000 IU/ml in their included AD patients[25]. They also found that IgE levels were increased above normal values in 92% AD patients. Other studies also reported that total serum IgE levels were increased in about 80% of AD patients especially those with sensitization to inhalant and food allergens, as well as associated chest allergies.[26, 27]

Consistent with our findings, a study by Mizawa et al., 2013, found no significant correlation between the SCORAD indices and serum IgE levels (p= 0.120). [28] On the other hand, Wu et al., 2011, revealed a positive correlation between SCORAD index and serum total IgE (p=0.028). [29] The difference between these studies might be explained by the effect of treatment (corticosteroids or antihistaminics) that influences serum levels of total IgE.

Studies have also found that polymorphisms in the NLRP3 gene are linked to AD.[30,31] NLRP3 deficient animals displayed lower skin inflammation and delayed disease initiation in a chronic proliferative dermatitis animal model, implying that the inflammasome could be a key driver for disease progression.[32] Moreover, Zheng et al., 2021, stated that compared to controls, the expression of NLRP3 mRNA was higher in skin lesion of AD patients.[33] Previous research demonstrated that inflammatory skin diseases trigger the NLRP3 inflammasome,[34] increasing the release of cytokines, which are crucial for host defense, inflammation, and immune system activation. [11] Additionally, since the NLRP3 inflammasome is crucial for the maturation and stimulation of dendritic cells, which are induced by MHC-II on macrophages as well as antigen-presenting cells, disruption of NLRP3 would compromise the immune response. [35]

Our study revealed that NLRP3 inflammasome rs10754558 G allele was more prevalent among AD patients (72.5%) than controls (53.2%). It increased the risk of AD by 2.33 folds (95% CI: 1.1-4.92, p=0.026). Furthermore, the rs10754558 G allele was associated with 7.17 folds (95% CI: 1.47 – 35.7) increased AD severity. Hitomi et al., 2009, reported that the G allele of the rs10754558 SNP altered the stability of NLRP3 mRNA and exhibited 1.3-fold greater activity than the C allele-specific construct.[8] In addition, the G allele may be associated with an increase in the serum level of interleukin (IL)-1β.[10]

Our study's limitations should also be noted. Firstly, the small size of its sample. Secondly, just one SNP (rs10754558) was examined, and the possibility of other genetic loci being involved in disease consequences could not be excluded. Thirdly, including only the Egyptian population. More research is needed on a wider diverse population and other NLRP3 polymorphisms to confirm the significance of the association between AD incidence and severity and NLRP3 genetic variations. In conclusion, NLRP3 inflammasome (rs10754558) G allele can be a significant factor predisposing to AD occurrence and severity.

Acknowledgements

We would like to express our gratitude to Dr. Shaimaa Hani Fouad from the Department of Internal Medicine's Allergy and Clinical Immunology for her assistance in making this work feasible.

Author Contributions

AG, FA, and OA designed the study and wrote the manuscript. MB and SB contributed to data collection. ST performed the statistical analysis and interpretation of the results. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (FMASU MS 54/2018).

Informed consent

A written informed consent was obtained from each participant before being included in the study, after explaining its purpose.

ORCID iD

Sara I. Taha **iD** https://orcid.org/0000-0001-8224-8701.

References

1. Mandlik, D. S., & Mandlik, S. K. (2021). Atopic dermatitis: new insight into the etiology, pathogenesis, diagnosis and novel treatment strategies. *Immunopharmacology and immunotoxicology*, *43*(2), 105–125.
2. Zhou, F., Li, C., & Zhang, S. Y. (2020). NLRP3 inflammasome: a new therapeutic target for high-risk reproductive disorders?. *Chinese medical journal*, *134*(1), 20–27.
3. Fang, X., Wang, Y., Zhang, Y., et al. (2021). NLRP3 Inflammasome and Its Critical Role in Gynecological Disorders and Obstetrical Complications. *Frontiers in immunology*, *11*, 555826.
4. Xiao, Y., Xu, W., & Su, W. (2018). NLRP3 inflammasome: A likely target for the treatment of allergic diseases. *Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology*, *48*(9), 1080–1091.
5. Wan, T., Pan, Q., & Ping, Y. (2021). Microneedle-assisted genome editing: A transdermal strategy of targeting *NLRP3* by CRISPR-Cas9 for synergistic therapy of inflammatory skin disorders. *Science advances*, *7*(11), eabe2888.
6. Paramel Varghese, G., Folkersen, L., Strawbridge, R. J., et al. (2016). NLRP3 Inflammasome Expression and Activation in Human Atherosclerosis. *Journal of the American Heart Association*, *5*(5), e003031.
7. Sahdo, B., Fransén, K., Asfaw Idosa, B., et al. (2013). Cytokine profile in a cohort of healthy blood donors carrying polymorphisms in genes encoding the NLRP3 inflammasome. *PloS one*, *8*(10), e75457.
8. Hitomi, Y., Ebisawa, M., Tomikawa, M., et al. (2009). Associations of functional NLRP3 polymorphisms with susceptibility to food-induced anaphylaxis and aspirin-induced asthma. *The Journal of allergy and clinical immunology*, *124*(4), 779–85. e6.
9. Zhang, Q. B., Qing, Y. F., He, Y. L., et al. (2018). Association of NLRP3 polymorphisms with susceptibility to primary gouty arthritis in a Chinese Han population. *Clinical rheumatology*, *37*(1), 235–244.
10. Zhou, D., Wang, X., Chen, T., et al. (2016). The NLRP3 rs10754558 Polymorphism Is Associated with the Occurrence and Prognosis of Coronary Artery Disease in the Chinese Han Population. *BioMed research international*, *2016*, 3185397.
11. Lee, Y. H., & Bae, S. C. (2016). Association between functional NLRP3 polymorphisms and susceptibility to autoimmune and inflammatory diseases: a meta-analysis. *Lupus*, *25*(14), 1558–1566.
12. Pontillo, A., Brandao, L., Guimaraes, R., et al. (2010). Two SNPs in NLRP3 gene are involved in the predisposition to type-1 diabetes and celiac disease in a pediatric population from northeast Brazil. *Autoimmunity*, *43*(8), 583–589.
13. Eichenfield, L. F., Tom, W. L., Chamlin, S. L., et al. (2014). Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. *Journal of the American Academy of Dermatology*, *70*(2), 338–351.
14. Baioumy, S.A., Sallam, D.E., Abdalgeleel, S.A., et al. (2021). Interleukin-17A serum levels in young patients with atopic dermatitis and food allergy. *European Cytokine Netw*ork. 32: 55–63.
15. Kim, J. H., Lee, S. Y., Kang, M. J., et al. (2018). Association of Genetic Polymorphisms with Atopic Dermatitis, Clinical Severity and Total IgE: A Replication and Extended Study. *Allergy, asthma & immunology research*, *10*(4), 397–405.
16. Cheng, L., Yin, R., Yang, S., et al. (2018). Rs4612666 Polymorphism of the NLRP3 Gene Is Associated with the Occurrence of Large Artery Atherosclerotic Ischemic Strokes and Microembolic Signals. *BioMed research international*, *2018*, 6345805.
17. Tanei, R., & Hasegawa, Y. (2016). Atopic dermatitis in older adults: A viewpoint from geriatric dermatology. *Geriatrics & gerontology international*, *16 Suppl 1*, 75–86.

18. Wey, G. D., Adefemi, S. A., & Amao, E. A. (2020). Prevalence and Pattern of Atopic Dermatitis among Children Aged 6 Months to 14 Years seen in General Out-Patient Clinic of Federal Medical Centre, Bida. *West African journal of medicine*, *37*(2), 124–130.
19. Bouman, A., Heineman, M. J., & Faas, M. M. (2005). Sex hormones and the immune response in humans. *Human reproduction update*, *11*(4), 411–423.
20. Xu, F., Yan, S., Li, F., Cai, M., Chai, W., Wu, M., Fu, C., Zhao, Z., Kan, H., Kang, K., & Xu, J. (2012). Prevalence of childhood atopic dermatitis: an urban and rural community-based study in Shanghai, China. *PloS one*, *7*(5), e36174. https://doi.org/10.1371/journal.pone.0036174
21. Sybilski, A. J., Zalewska, M., Furmańczyk, K., et al. (2015). The prevalence of sensitization to inhalant allergens in children with atopic dermatitis. *Allergy and asthma proceedings*, *36*(5), e81–e85.
22. Andersen, L., Nyeland, M. E., & Nyberg, F. (2020). Increasing severity of atopic dermatitis is associated with a negative impact on work productivity among adults with atopic dermatitis in France, Germany, the U.K. and the U.S.A. *The British journal of dermatology*, *182*(4), 1007–1016.
23. Yano, C., Saeki, H., Ishiji, T., et al. (2013). Impact of disease severity on work productivity and activity impairment in Japanese patients with atopic dermatitis. *The Journal of dermatology*, *40*(9), 736–739.
24. Kantor, R., Kim, A., Thyssen, J. P., et al. (2016). Association of atopic dermatitis with smoking: A systematic review and meta-analysis. *Journal of the American Academy of Dermatology*, *75*(6), 1119–1125.e1.
25. Sehgal, V. N., Srivastava, G., Aggarwal, A. K., et al. (2015). Atopic Dermatitis: A Cross-Sectional (Descriptive) Study of 100 Cases. *Indian journal of dermatology*, *60*(5), 519.
26. Johnson, E. E., Irons, J. S., Patterson, R., et al. (1974). Serum IgE concentration in atopic dermatitis. Relationship to severity of disease and presence of atopic respiratory disease. *The Journal of allergy and clinical immunology*, *54*(2), 94–99.
27. Akdis, C. A., Akdis, M., Bieber, T., et al. (2006). Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. *The Journal of allergy and clinical immunology*, *118*(1), 152–169.
28. Mizawa, M., Yamaguchi, M., Ueda, C., et al. (2013). Stress evaluation in adult patients with atopic dermatitis using salivary cortisol. *BioMed research international*, *2013*, 138027.
29. Wu, K. G., Li, T. H., Chen, C. J., et al. (2011). Correlations of serum Interleukin-16, total IgE, eosinophil cationic protein and total eosinophil counts with disease activity in children with atopic dermatitis. *International journal of immunopathology and pharmacology*, *24*(1), 15–23.
30. Macaluso, F., Nothnagel, M., Parwez, Q., et al. (2007). Polymorphisms in NACHT-LRR (NLR) genes in atopic dermatitis. *Experimental dermatology*, *16*(8), 692–698.
31. Zhang, Q., Fan, H. W., Zhang, J. Z., et al. (2015). NLRP3 rs35829419 polymorphism is associated with increased susceptibility to multiple diseases in humans. *Genetics and molecular research: GMR*, *14*(4), 13968–13980.
32. Douglas, T., Champagne, C., Morizot, A., et al. (2015). The Inflammatory Caspases-1 and -11 Mediate the Pathogenesis of Dermatitis in Sharpin-Deficient Mice. *Journal of immunology (Baltimore, Md.: 1950)*, *195*(5), 2365–2373.
33. Zheng, J., Yao, L., Zhou, Y., et al. (2021). A novel function of NLRP3 independent of inflammasome as a key transcription factor of IL-33 in epithelial cells of atopic dermatitis. *Cell death & disease*, *12*(10), 871.
34. Bitto, A., Altavilla, D., Pizzino, G., et al. (2014). Inhibition of inflammasome activation improves the impaired pattern of healing in genetically diabetic mice. *British journal of pharmacology*, *171*(9), 2300–2307.
35. Ogura, Y., Bonen, D. K., Inohara, N., et al. (2001). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature*, *411*(6837), 603–606.