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The Relationship between TLR2 SNP Polymorphism and IL10, IL6 Levels in Atopic Dermatitis Patients

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Abstract

Inflammatory diseases like atopic dermatitis have shown a correlation between changes in immune responses and variations in the *TLR2* gene, with a specific focus on how *TLR2* polymorphisms influence *IL-10* and *IL-6* levels in individuals with AD. Fifty eight patients with atopic dermatitis and 32 healthy controls provided blood samples for analysis. *IL-10* and *IL-6* levels were measured using Elisa assay, and *TLR2* gene variants were identified with Sanger sequencing. The results revealed that *TLR2* polymorphisms, particularly (T-6686-A), were not significantly associated with AD patients compared to healthy controls. There was a significant increase in the *IL-6* and *IL-10* levels between atopic dermatitis patients and healthy control. Otherwise, the result showed a significant increase in the *IL-6* level and no significant increase in the *IL-10* level between AD patients' men and women. These results suggest a potential link between *TLR2* genetic variations and altered *IL-10* or *IL-6* levels in AD patients, highlighting the influence of *TLR2* polymorphisms on immune responses in this condition. Studying *TLR2* polymorphisms' effect on cytokine profiles could offer insights into AD pathogenesis and guide personalized immune-targeted therapies for affected individuals. The absence of significant differences in the distribution of genotypes among various allele variants may be due to the small size of the sample or the variant *TLR2* (rs4696480). Individuals with AD often exhibit elevated levels of *IL-6* and *IL-10*, highlighting the complex immune dysfunction associated with this condition.

Keywords: polymorphism, *S. aureus*, Atopic Dermatitis, *TLR2, IL-10* and *IL-6.*

Резюме

Възпалителни заболявания като атопичен дерматит са показали връзка между промените в имунните отговори и вариациите в гена TLR2, със специфичен фокус върху това как полиморфизмите на TLR2 влияят върху нивата на IL-10 и IL-6 при индивиди с AD. Петдесет и осем пациенти с атопичен дерматит и 32 здрави контроли предоставиха кръвни проби за анализ. Нивата на IL-10 и IL-6 бяха измерени с помощта на тест Elisa, а вариантите на гена TLR2 бяха идентифицирани със секвенирането на Sanger. Резултатите показват, че TLR2 полиморфизмите, особено (T-6686-A), не са значително свързани с пациенти с AD в сравнение със здрави контроли. Има значително увеличение на нивата на IL-6 и IL-10 между пациенти с атопичен дерматит и здрави контролни групи. В противен случай резултатът показва значително увеличение на нивото на IL-6 и няма значително увеличение на нивото на IL-10 между мъже и жени на пациенти с AD. Тези резултати предполагат потенциална връзка между генетичните вариации на TLR2 и променените нива на IL-10 или IL-6 при пациенти с AD, подчертавайки влиянието на полиморфизмите на TLR2 върху имунните отговори при това състояние. Изследването на ефекта на полиморфизмите на TLR2 върху цитокиновите профили може да предложи обяснение за патогенезата на AD и да насочи персонализирани имуно-насочени терапии за засегнатите индивиди. Липсата на значителни разлики в разпределението на генотипите между различните алелни варианти може да се дължи на малкия размер на пробата или на варианта TLR2 (rs4696480). Индивидите с AD често показват повишени нива на IL-6 и IL-10, подчертавайки сложната имунна дисфункция, свързана с това състояние.

Introduction

Atopic Dermatitis (AD), often called eczema, is a long-term inflammatory skin condition recognized by symptoms such as dry skin, persistent itching, and recurrent eczema lesions (Langan

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et al., 2020; Jafer and Mahmood, 2022). It affects a considerable portion, approximately 20% of children and around 2% to 10% of adults globally. The impact of AD goes beyond the physical symptoms, profoundly influencing the quality of life for patients and their families, and it can pose financial burdens as well (Mucha *et al.,* 2020). AD is linked to various genes, including innate/adaptive immune systems, *HLA*, cytokines, chemokines, drug-metabolizing genes, and other related factors (Al-Shobaili *et al.,* 2016). The pathophysiology of Atopic Dermatitis (AD) is intricate, involving a combination of factors, including a solid hereditary predisposition, dysfunction of the skin's outer layer (epidermis), and inflammation driven by T-cells (Langan *et al.,* 2020). The skin's microbiome, plays a crucial role in AD pathogenesis. *Staphylococcus aureus* is a prominent organism with a well-known ability to cause various infections both in clinical and community settings (Uzeh *et al.,* 2023). AD patients exhibit an imbalance in skin microbial composition, marked by decreased microbial variety and an increased presence of the pathogenic bacterium *Staphylococcus aureus* (Koh *et al.,* 2022; Zina and Alubydi, 2022). An overgrowth of *Staphylococcus aureus* could be observed in 30-100% AD patients (Paller *et al.,* 2018). The capability of *S. aureus* to tackle the skin's natural defenses using their virulence factors could be the cause of this overgrowth. *S. aureus* fibronectin shows a particular affinity for type 2 inflammation, as observed in recent studies (Andrea *et al.,* 2019; Wang *et al.,* 2020; Farag *et al.,* 2022). Moreover, superantigens produced by *S. aureus* have the ability to breach the skin's protective layer and initiate T-helper 2 (TH2) inflammation. This increased exposure to microbial products might lead to elevate percutaneous sensitization, initiating a detrimental cycle that further stimulates the host's immune response, consequently exacerbating AD symptoms (Lunjani *et al.,* 2018; Wang *et al.,* 2020).

Toll-like receptors (*TLRs*) are integral to initiating immune responses by controlling the synthesis of inflammatory cytokines and antimicrobial peptides (Talak and Ghaima*,* 2023). These receptors, evolutionary conserved transmembrane proteins, recognize specific patterns associated with pathogens. Upon detecting these patterns, *TLRs* trigger downstream signaling pathways, inducing cytokine production and activating immune cells. *IL-6* and *IL-10* are pivotal cytokines involved in various aspects of immune cell activation, differentiation, inflammation, and cell growth (Kumar, 2020). Polymorphisms within genes encoding Toll-like receptors, such as TLR2, can compromise the body's immune response and increase vulnerability to bacterial infections (Mozyrska, 2022). Specific single nucleotide polymorphisms (SNPs) in TLR genes, like rs187084 and rs5743551, have been linked to heightened susceptibility to diverse infections, including tuberculosis, malaria, and sepsis (Wurfel *et al.,* 2008; Bronkhorst *et al.,* 2015). These genetic variations correlate with alterations in TLR signaling pathways, resulting in reduction of cytokine production and impaired immune responses (Selvaraj *et al.,* 2015; Bronkhorst *et al.,* 2015). Overall, SNPs within TLR genes have been associated with modified immune responses and an augmented susceptibility to various infectious and inflammatory diseases. Further exploration is crucial to fully comprehend the mechanisms underlying these associations and their potential implications in disease prevention and treatment (Lucarelli *et al.,* 2016).

This research investigates the connection between a specific TLR2 gene variant (rs4696480) and the levels of IL-6 and IL-10 in Iraqi individuals with atopic dermatitis, which is the first study of its kind in Iraq. The goal of this study is to gain a deeper understanding of how genetic differences in the TLR2 gene may impact the susceptibility to atopic dermatitis, as well as the regulation of key cytokines involved in immune responses in this condition. By examining how variations in the TLR2 gene can affect cytokine levels, valuable insights into the development of atopic dermatitis may be uncovered, potentially leading to personalized treatment strategies tailored to individuals' genetic makeup.

Materials and Methods

Sample collection

This study was conducted at the Dermatology Centre at Medical City, Kadhimiya Educational Hospital, and Yarmouk Educational Hospital from July 2022 to January 2023. A total of 90 blood samples were collected from 58 patients suffering from atopic dermatitis skin inflammation caused by *S. aureus* and 32 healthy controls with different ages and gender. Five ml of venous blood was collected using a disposable syringe from each participats (patients and control). The blood was divided into two portions, three ml were transferred to gel tubes for serum collection and tow ml collected in an ethylene diamine tetra acetic acid (EDTA) tube for DNA extraction. The serum was separated into gel

tube by centrifuging for 10 minutes at 3000 rounds per minute (rpm); the serum was preserved in 1.5 ml Eppendorf tubes and kept in the 20ºC for further analysis steps. *S. aureus* were isolates from all patients and identified with biochemical tests and via VITEK 2 (BioMérieux, Marcy-l'Étoile, France).

Estimation of the cytokine levels

To estimate the levels of human *IL-6 and IL-10,* the Enzyme Linkage Immune Sorbent Assay has been performed using Fine Test (Wuhan Fine Biotech Co., China). The kit depends on the sandwich enzyme-linked immune-sorbent assay technique. A 96-well plate pre-coated with capture antibody, and a biotin-conjugated antibody was utilized as a detection antibody. The wells were filled with the standards, test samples, and biotin-conjugated detection antibody before being cleaned with wash buffer. After adding HRP-Streptavidin, unbound conjugates were removed using a wash buffer. The HRP enzymatic reaction was seen using TMB substrates. TMB was catalyzed by HRP to provide a blue product, which became yellow upon the addition of an acidic stop solution. The goal amount of sample caught in the plate is directly correlated with the yellow density. Using a microplate reader, read the O.D. absorbance at 450 nm to compute the target concentration and change the color orange. The optical density (OD) was measured at 450 nm using a spectrophotometer.

Detection of SNPs for the TLR2 (T-6686-A) SNP

Total genomic DNA was extracted from blood samples using (New England Biolabs / UK) kit. The results of DNA extraction showed that fresh blood samples yielded enough DNA concentration for PCR amplification. The concentration and purity of DNA measured by NanoDrop (thermo-fisher scientific) revealed that the DNA concertation ranged between 0.2- 1.00 ng /µl and the purity was ranged between 1.7-2.

Conventional PCR were applied to amplify the specific region of the SNPs, the primers were designed for this SNPs using ((NCBI) National Centre for Biotechnology Information PRIMER BLAST Approach). The forward primer F: 5'-GTTCTTCTTGTTCTAAGCAAG-3', and the reverse was R: 5'-GGTGATTAGGTTATGAAGGCT-3'. The PCR reaction mix was 25 µl and consisted of 12.5 µl of ready-to-use PCR master mix (New England Biolabs / UK) 3 µl of template DNA and 1.5 µl (15 pmol) of each primer. The rest of the volume was completed with nuclease-free water. The mixtures were subjected to the following thermal cycling parameters in a thermocycler (New et al. / UK): Initial denaturation at 94°C for 5 min, followed by 30 cycles of amplification at 94°C for 45 sec, annealing at 48 °C for 45 sec. The PCR product was sent to Macrogen Corporation – Korea for sanger sequencing. The results were analyzed using Genius software.

Statistical analysis

The chi-squared (χ^2) equation is a statistical test used to determine whether there is a significant difference between the expected frequencies and the observed frequencies of rs (4696480) snp. If the calculated χ^2 value is greater than the critical value for a given significance level (usually 0.05), then the null hypothesis of HWE is rejected, indicating that the population is not in Hardy-Weinberg equilibrium.

Result

In order to detect the relationship between the immune response and the symptoms development in AD patients, the level of *IL-6* and *IL-10* was estimated in patients' serum and compared with controls. The result revealed that the mean levels of *IL-6* and *IL-10* are significantly elevated in AD patients in compare with the healthy once were (193.87 ±5.62 *vs.*83.98 ±6.34 pg/ml) with (p-value = 0.0001, T-test =17.733) for *IL-6* and (549.46 ±25.95 *vs.*312.19 ±7.01 pg/ml) with (p-value = 0.0001, T-test =70.374) for *IL-10*. Table 1. Interestingly, a considerable increase in *IL-6* levels was observed between male and female AD patients (183.48 ±6.43 *vs.*206.67 ±9.27 pg/ml) with (p-value = 0.0313, T-test =21.045) while no significant difference was found in *IL-10* levels among these gender groups were (562.84 ±39.96 *vs.*533.01 ±31.12 pg/ml) with (p-value = 0.578, T-test =106.92), Table 2.

Table 1. Comparison between patients and healthy control groups in *IL-6* and *IL-10*

Group	Mean ± SE	
	IL-6 (pg/ml)	IL-10 (pg/ml)
Patients	193.87 ±5.62	549.46 ±25.95
Control	83.98 ±6.34	312.19 ±7.01
T-test	17.733 **	70.374 **
P-value	0.0001	0.0001

** (P≤0.01)

Genotyping of single nucleotide polymorphism of TLR2 gene (rs4696480)

To detect the distribution of rs4696480 genotype of TLR-2 gene among the AD patients, a 545 bp segment of has been amplified using

conventional PCR techniques (Fig. 1), and sequenced using sanger methods.

Table 2. Relationship between gender with *IL-6* and *IL-10* in patients' group

Gender	Mean ± SE	
	IL-6 (pg/ml)	IL-10 (pg/ml)
Male	183.48 ±6.43	562.84 ±39.96
Female	206.67 ±9.27	533.01 ±31.12
T-test	21.045 *	106.92 NS
P-value	0.0313	0.578

** (P≤0.01)

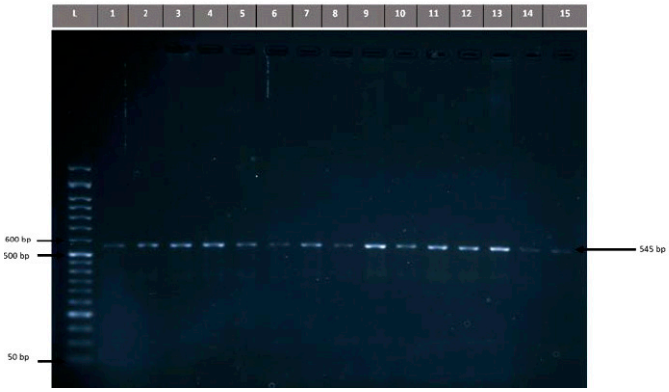

Fig 1. Agarose Gel Electrophoresis of PCR Amplified Products for *TLR2* (rs4696480)Gene. Lane (L): 50 bp ladder, lanes (1-15): DNA with positive result with expected size 545 bp (2% Agarose, 80 V for 70 min)

TLR2((rs4696480) (T-6686-A)) polymorphisms, genotype distribution, and allele frequency concerning AD risk in Iraqi patients

The analysis of the sequencing results of the SNP ((rs4696480) (T>A)) inside *TLR2* illustrated that the genotype frequencies for both the control and the patients were found to follow Hardy-Weinberg equilibrium (HWE). To assess the agreement between the observed and expected genotype frequencies, each genotype distribution was compared in both groups. The result revealed no-significant for each genotype between the patients and control P-values = 0.9240 for patients and 0.4067 for controls (Table 3). This indicates that there is no significant deviation in the expected genotype frequencies for both groups, supporting the assumption of genetic equilibrium within this study populations.

The distribution of genotypes is outlined in Table 4, showing that the AD the genotypic distribution for TT was (37.1% , 40.63%) for patients and control respectively, the p- value was (0.829%) which indicate there was a non-significant decrease in the percentage of TT in patients compared to healthy people, for TA was (48.1% , 40.63%) for patients and control respectively, the p- value was (0.512%) there was a non-significant increase in the percentage of TA in patients compared to control , and for AA was (14.8% , 18.75%) for patients and control respectively, the p- value was (0.764%) there was a non-significant decrease in the percentage of AA in patients compared to control. The ratio between A and T alleles are equal between two groups. otherwise, the odd ratio (OR) percentage for TT and AA appeared as a protective factor against (AD) disease duo of the (OD) value was (0.108 and 0.228) less than 1, while the (OR) for TA was (1.36) higher than 1 This indicates that it is a risk factor for a person have this genotype allele.

Discussion:

Atopic dermatitis in another word Atopic Eczema is a chronic inflammatory skin disease that effecting all ages, described by lesions of eczematous and acute itch (Jassim and Al-Kazaz, 2023). It is one of the diseases that is related directly to human immune system disturbances. It is caused as a result of different etiological factors most of which are correlated with skin immunology and skin microbiome that may related to the type of child delivery (Muhanad and Al-Aubydi, 2020). Toll-like receptors (TLRs), especially *TLR2*, play a crucial role in

Table 3. Hardy-Weinberg equilibrium to determine the significant difference between the expected frequencies and the observed frequencies

	TLR2 (rs4696480)			
	Patients (54) No. (%)		Control (32) No. (%)	
	Observed	Expected	Observed	Expected
TT	20 (37.04)	20.17 (37.35)	13 (40.63)	11.88 (37.13)
TA	26 (48.15)	25.67 (47.53)	13 (40.63)	15.23 (47.61)
AA	8 (14.81)	8.17 (15.12)	6 (18.75)	4.88 (15.26)
Total	54 (100.0)	54 (100.0)	32 (100.0)	32 (100.0)
P- value	0.9240		0.4067	

p- value: p-value of Hardy-Weinberg equilibrium

Table 4. Genotype distribution and allele frequency of TLR2 gene polymorphism (rs4696480) in patients and control groups

	TLR2 (rs4696480)				
Genotyping	Patients (54) No. (%)	Control (32) No. (%)	X^2	OR (95% CI)	*P*- value
TT	20 (37.1)	13 (40.63)	0.108	0.86 (0.36-2.08)	0.820
TA	26 (48.1)	13 (40.63)	0.454	1.36 (0.57-3.25)	0.512
AA	8 (14.8)	6 (18.75)	0.228	0.75 (0.24-2.37)	0.764
Total	54 (100.0)	32 (100.0)			
Alleles frequency					
T	66 (61.0)	39 (61.0)	0.001	1.01 (0.54-1.89)	1.0
A	42 (39.0)	25 (39.0)	0.001	0.99 (0.53-1.86)	1.0

p-value: Fisher's exact probability

recognizing both external threats like bacteria as well as internal danger signals associated with inflammation and tissue damage. In individuals with AD, there is often significant bacterial colonization by pathogens like *S. aureus*, which can activate *TLR2* and exacerbate immune responses (Kim *et al.,* 2016).

This study focused on exploring the association between *TLR2* genotype and *IL10, IL6* levels in atopic dermatitis patients with skin inflammation caused by *S. aureus* in Iraqi individuals, which has not been previously studied. The study specifically investigated a single nucleotide polymorphism (SNP) in the *TLR2* gene, known as (rs4696480), among participants from the Baghdad region of Iraq across different age brackets. The findings revealed that *TLR2* gene variations were equally prevalent among both AD patients and the control group.

It has been determined that *Staphylococcus aureus* can penetrate the skin barrier and cause both acute and chronic inflammation in the skin, which in turn affects the severity and course of atopic dermatitis (AD). According to Kim *et al.* (2019) and Esposito *et al.* (2017) *S. aureus* colonizes up to 90% of AD patients, making it more common in AD than in other illnesses. The degree of *S. aureus* skin colonization in AD patients is mostly determined by environmental factors and the individual's immunological condition (Mozyrska *et al.,* 2022).

Toll-like receptor 2 is able to recognize *S. aureus*, which is known to colonize skin lesions in AD patients, because of the components of its cell wall (Elson *et al.,* 2007). Studies indicate that AD patients have impaired *TLR2*-mediated immunological signaling pathways, with reduced *TLR2* expression on Langerhans cells (LC) in AD patients with significant *S. aureus* colonization. Reduced LC maturation and migration activity as well as decreased *IL-6* and *IL-10* production were linked to this compromised *TLR2* signaling in skin samples from AD patients (Iwamoto *et al.,* 2018).

Moreover, during both the acute and chronic phases of AD, macrophages, which also express *TLR2* accumulate in skin lesions. When exposed to *TLR2* ligands, macrophages from peripheral blood monocytes of AD patients produced less pro-inflammatory cytokines such as *IL-6, IL-8*, and *IL-1β* than did healthy controls (Niebuhr *et al.,* 2009). All of these findings point to the possibility that compromised *TLR2*-mediated immune responses, in particular *S. aureus* colonization in afflicted skin regions, may exacerbate inflammation and the severity of AD. Niebuhr et al. reported diminished responses in keratinocytes from AD patients when exposed to *TLR2* agonists. This reduced response manifested as lower production of various inflammatory molecules like *IL-6, IL-8, CCL20*, and matrix metalloproteinase-9 (*MMP-9*) (Niebuhr *et al.,* 2011). Another study by Naji and Mousa conducted a study in Nasiriyah, Iraq, where they observed elevated serum levels of *IL-6* in AD patients compared to control groups. Additionally, they noted significantly higher levels of *IL-6* in severe AD patients compared to the control group, indicating a correlation between elevated *IL-6* levels and the clinical severity of AD. This suggests a critical role for *IL-6* in the pathogenesis of dermatitis, particularly in AD (Naji and Mousa, 2022).

Hayes *et al*. (2017) also reported increased levels of *IL-6* in the serum of adult patients affected by atopic dermatitis, asthma, and eczema. Oh *et al*. (2009) highlighted the relevance of *TLR2* in severe AD among adults; they found a significant increase in the representation of the A-allele in the *TLR2* (rs4696480) gene variant among severe AD patients. This finding aligns with the notion that

IL-6 may be involved in the immune dysregulation observed in various allergic and inflammatory conditions, including AD. The stimulation assays revealed a reduction in *IL-6* secretion but not in *TNF-α, the* A allele homozygous carriers of *TLR2* (rs4696480) (Oh *et al.,* 2009). In addition, the stimulation of TLR2 enhances *IL-10* secretion by keratinocytes in AD patients. (Jeong *et al.,* 2003) reported ongoing debates among researchers studying *IL-10* secretion by these cells.

Tyurin *et al*. (2017) have observed a significant increase in *IL-10* levels in patients with AD possessing the heterozygous genotype of a specific polymorphism, suggesting a potential association between *IL-10* and AD pathogenicity. A previous study by Asadullah et al. conducted in murine models and analyzing patient expression data indicated the significant impact of *IL-10* on various inflammatory, cancer, and autoimmune diseases, highlighting its regulatory role in modulating disease courses (Asadullah *et al.,* 2003). In inflammatory conditions like psoriasis and atopic dermatitis, skin-associated B cell subsets play specific roles. They make antibodies, communicate with skin T cells, form tertiary lymphoid tissue, make pro-inflammatory cytokines, and even suppress the immune system by releasing IL-10 (Debes and McGettigan, 2019). Another study by Suga and Sato demonstrated a significant reduction in B cells's *IL-10* secretion among patients with these skin conditions, suggesting a potential role for regulatory B cells in disease suppression. Dysfunction in these cells could exacerbate disease symptoms (Suga and Sato, 2019).

Upon analyzing clinical samples from psoriasis patients, Hayashi et al. observed a reduction in regulatory B cells that produce *IL-10*. They did not, however, discover a connection between the number of these cells and the psoriasis disease severity score (Hayashi *et al.,* 2016). Suga and Sato (2019) looked at how *IL-10*-producing B cells affected the production of IgE in AD mice and discovered that these cells were not as effective as they had been thought. In the AD group, there was a significant drop in the number of B cells that produced *IL-10*, and these cells showed impaired regulatory capabilities. This implied that the decreased frequency and compromised function of these cells could exacerbate allergic illnesses like AD by causing uncontrollably high levels of allergic inflammation (Suga and Sato, 2019).

In addition, Hayashi *et al.* (2016) discovered that individuals with severe AD had fewer B cells that produce *IL-10* when compared to individuals with mild AD or healthy controls. They observed a negative relationship between the number of these cells and blood CCL17 levels in AD patients as well as skin scores for the severity of the condition. This implies that the degree of allergic inflammation in AD may be correlated with the diminished frequency and compromised functionality of *IL-10*-producing B cells, underscoring their possible involvement in the course of the illness (Hayashi *et al.,* 2016).

Debinska *et al.* (2019) examined the genotype frequencies of the *TLR2* (rs4696480) single nucleotide polymorphism. They reported frequencies of 38.8% for TT, 37.3% for TA, and 23.9% for AA genotypes; they also found the A allele frequency to be 42.5%. However, their result did not reveal any significant differences in either genotype or allele frequencies of the *TLR2* (rs4696480) polymorphism between AD children and controls (Dębińska *et al.,* 2019). The single nucleotide polymorphism *TLR2* (rs4696480) has genotype frequencies of TT, TA, and AA, respectively, according to a study by Can et al. within the atopic dermatitis (AD) group, they discovered that the frequency of the T allele was 46.43% and that of the A allele was 53.57%. They found no statistically significant variations in the distribution of these polymorphisms between the groups in their research, suggesting that the study population's vulnerability to AD may not be much influenced by this particular *TLR2* variant (Can *et al.,* 2017). In contrast, Salpietro et al. examined *TLR2* (rs4696480) SNPs in 150 healthy children and 187 children with atopic dermatitis compared to controls. They found that AD patients had a greater frequency of the homozygous A/A genotype, but they were unable to discover a connection between the severity of atopic dermatitis and the *TLR2* A gene (Salpietro *et al.,* 2011). This suggests that while the genotype frequencies of AD patients and controls may vary, the specific allele (the A allele) may not directly correlate with the severity of the disease (Mozyrska *et al.,* 2022). According to a large European study by Eder et al. children of farmers who had the T allele in *TLR2* (rs4696480) were less likely to have symptoms of hay fever, asthma, or atopic sensitization found in them. This suggests that this genetic variant may have a protective impact on this population (Eder *et al.,* 2004). On the other hand, the *TLR2* (rs4696480) polymorphism was not observed to be significantly correlated with the development of atopy or asthma/allergic rhinitis; for example, research by Lam et

al. on farmers in Denmark did not show that *TLR2* polymorphisms affected the likelihood of atopy and new-onset asthma (Lam *et al.,* 2004). Furthermore, Ortiz-Martinez et al. found no connection between asthma and the *TLR2* (rs4696480) polymorphism in their investigation of the Puerto Rican population (Ortiz-Martinez *et al.,* 2016).

Children with at least one A allele in *TLR2* (rs4696480) had a significantly lower chance of having asthma identified by a physician when compared with (AD) children who hold the AA genotype of the *TLR2* gene (rs4696480) and had a considerably increased chance of carrying *S. aureus* (Kang *et al.,* 2010; Mozyrska *et al.,* 2022). Other study reported that the T allele also provides some protective benefits examined a number of medical disorders and proposed a link between (rs4696480) and mouth cancer in Caucasians (De Barros *et al.,* 2017). In contrast, previous study by Semlali et al. has been indicated that there was no statistically significant variation in the expression of (rs4696480) between Asian breast cancer patients and controls (Semlali *et al.,* 2018). In addition, the frequency of *TLR2* (rs4696480) polymorphisms in patients in order to investigate any potential relationship between these polymorphisms and nasal carriage of *S. aureus*. This research, however, did not uncover any connection between the (rs4696480) polymorphism and nasal carriage of *S. aureus* (Zukowski *et al.,* 2017).

In AD, a notable elevation of *IL-6* compared to *IL-10* indicates a distinct pro-inflammatory milieu. This imbalance likely contributes to the condition's persistent inflammation and characteristic symptoms. The variability observed in *IL-6* and *IL-10* levels among AD patients underscores this disease's complexity of immune dysregulation (EL-Aal *et al.,* 2016). Understanding the interplay between pro-inflammatory like *IL-6* and anti-inflammatory like *IL-10* cytokines is critical for unraveling the pathogenesis of AD and devising targeted treatments that effectively modulate these immune responses. Higher levels of both *IL-6* and *IL-10* might correlate with more severe forms of AD (Lee *et al.,* 2000; Israeli *et al.,* 2022). Monitoring the levels of these cytokines could serve as markers to assess disease severity, aiding in clinical management and treatment decisions for individuals with AD (Yu and Li, 2022).

Conclusion

According to this research, there is no relationship between AD and the *TLR2* (rs4696480) SNPs that have been detected. However, additional statistical analysis was needed to assess the association between *TLR2* (rs4696480) SNPs, total *IL6, IL10* levels, and the severity of AD, as the polymorphisms were distributed equally in patients and controls. The lack of substantial variations in the genotype distribution for all allele variants could be attributed to the variant *TLR2* (rs4696480) or the limited sample size.

The intricacy of immunological dysregulation in AD is reflected in elevated levels of *IL-6* and *IL-10* in patients. The resistive dysregulation and persistent inflammation that characterize AD may be influenced by inflammation.

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