

RESEARCH ARTICLE

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Association of mannose-binding lectin 2 (rs11003125) gene polymorphism and serum levels with atopic dermatitis in Iraqi patients

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Abstract

Objective: Atopic dermatitis (AD) is a chronic inflammatory skin disease with complex pathogenesis involving genetic and immune factors. This study investigated the association between the MBL2 rs11003125 polymorphism, serum mannose-binding lectin (MBL) concentrations, and AD susceptibility in an Iraqi cohort.

Methods: This case-control study included 60 clinically diagnosed AD patients and 40 age- and sex-matched healthy controls. Serum MBL levels were quantified by ELISA. Genomic DNA was extracted from blood, and the MBL2 rs11003125 polymorphism was genotyped by PCR and Sanger sequencing. Statistical analyses included chi-square, Mann-Whitney U, Kruskal-Wallis, and multivariate logistic regression.

Results: Serum MBL levels were significantly lower in AD patients (median 84.42 µg/L, interquartile range [IQR]: 42.59–139.79 µg/L) than in controls (median 147.62 µg/L, IQR: 49.65–526.49 µg/L; $p = 0.005$). The C allele of rs11003125 was associated with increased AD risk (odds ratio [OR] = 2.18, 95% confidence interval [CI]: 1.22–3.90, $p = 0.006$). In a dominant model, individuals carrying at least one C allele (GC+CC) demonstrated a 3.9-fold increased AD risk (adjusted OR = 3.9, 95% CI: 1.5–9.2, $p = 0.004$). Serum MBL levels showed significant genotype-dependent reduction, with the highest levels in GG (median 133.49 µg/L), intermediate in GC (median 87.58 µg/L), and lowest in CC (median 36.68 µg/L; $p = 0.0004$).

Conclusions: The MBL2 rs11003125 polymorphism was significantly associated with reduced serum MBL levels and conferred increased risk of atopic dermatitis in the studied Iraqi population, highlighting the role of MBL-mediated innate immunity in AD pathogenesis.

Keywords: atopic dermatitis; mannose-binding lectin; genetic polymorphism; Iraq; innate immunity

Plain English Summary

Atopic dermatitis, also known as eczema, is a long-lasting skin condition that causes itchy, inflamed skin. While we know that both genes and the immune system play important roles in this condition, we do not fully understand all the factors involved. This study looked at whether a specific genetic variation (called rs11003125) in the gene that produces mannose-binding lectin (MBL), a protein that helps the body fight germs, affects the risk of developing atopic dermatitis in Iraqi people. We compared 60 patients with atopic dermatitis to 40 healthy volunteers of similar ages and genders. We found that patients with atopic dermatitis had lower levels of MBL in their blood than healthy people. We also discovered that a particular version of the MBL gene (the C allele) was more common in patients and was linked to lower MBL levels. People carrying this genetic variation had nearly four times higher risk of having atopic dermatitis. These results suggest that the body's natural ability to fight germs through MBL may be important in protecting against atopic dermatitis. This knowledge could help us better understand why some people develop this condition and may guide future approaches to treatment or prevention.

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Introduction

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disorder that affects individuals across all age groups, from early childhood to older adulthood. The condition is characterised by intense pruritus, eczematous lesions, skin barrier dysfunction, and significant impairment in quality of life (1). The global prevalence of AD has increased substantially over recent decades, with estimates suggesting that 15–20% of children and 1–3% of adults are affected worldwide (2). The pathogenesis of AD is complex and multifactorial, involving intricate interactions between genetic predisposition, immune dysregulation, environmental factors, and skin microbiome alterations (3).

While considerable research has focused on the role of adaptive immunity, particularly T-helper 2 (Th2) cell-mediated responses, in AD pathogenesis, the contribution of the innate immune system is increasingly recognised as a critical component in disease initiation and perpetuation (4). The compromised skin barrier in AD patients allows enhanced penetration of allergens and microbes, triggering innate immune responses that can exacerbate inflammation and create a self-perpetuating cycle of barrier disruption and immune activation (5).

Mannose-binding lectin (MBL) is a pattern-recognition molecule primarily synthesised in the liver and belongs to the collectin family of proteins. As a soluble component of the innate immune system, MBL recognises and binds to carbohydrate patterns expressed on the surfaces of various microorganisms, including bacteria, viruses, fungi, and parasites (6). Upon binding, MBL activates the lectin pathway of the complement system through associated serine proteases, promoting opsonisation, phagocytosis, and direct microbial killing (7). Beyond its role in antimicrobial defence, MBL also participates in the clearance of apoptotic cells and modulation of inflammatory responses, positioning it as a crucial molecule at the interface of innate and adaptive immunity (8).

The *MBL2* gene, located on chromosome 10q11.2-q21, encodes the human MBL protein. Several single-nucleotide polymorphisms (SNPs) in both the promoter region and exon 1 of *MBL2* have been shown to significantly influence circulating MBL concentrations and functional activity (9). Among these, the rs11003125 polymorphism (also known as -550 G/C or H/L variant) in the promoter region affects gene transcription efficiency, with the C allele associated with reduced promoter activity and consequently lower serum MBL levels (10). Considerable inter-individual and inter-population variability exists in MBL concentrations, largely attributable to these

genetic variations, with MBL deficiency representing one of the most common primary immunodeficiencies worldwide (11).

The relationship between MBL deficiency and susceptibility to infectious diseases is well-established, particularly during critical windows of immune system development when adaptive immunity is still maturing (12). However, emerging evidence suggests that MBL may also play a role in inflammatory and allergic conditions. Several studies have reported associations between *MBL2* polymorphisms and various diseases, including systemic lupus erythematosus, rheumatoid arthritis, cystic fibrosis, and recurrent infections (13). In the context of allergic diseases, MBL deficiency has been linked to increased sensitisation to microbial antigens and more severe disease manifestations (14).

The potential involvement of MBL in AD pathogenesis is biologically plausible through multiple mechanisms. First, MBL deficiency may impair host defence against *Staphylococcus aureus*, which colonises the skin of up to 90% of AD patients and is known to exacerbate disease through superantigen production and barrier disruption (15). Second, MBL-mediated complement activation may influence the Th2-polarised immune response characteristic of AD, as complement components have been shown to modulate T-cell differentiation and function (16). Third, MBL's role in apoptotic cells and debris may affect the resolution of inflammation in chronically inflamed skin (17).

Despite this biological rationale, studies investigating the association between *MBL2* polymorphisms and AD have yielded inconsistent results across different populations. A study in a Korean population reported associations between specific *MBL2* haplotypes and AD susceptibility (18), while investigations in European cohorts have shown variable findings (19). To date, no comprehensive study has examined this relationship in an Iraqi population, which possesses a distinct genetic background and environmental exposures that may influence both MBL expression and AD pathogenesis. The prevalence and characteristics of AD in Iraqi populations have been described in previous epidemiological work (20, 21), highlighting the need for population-specific genetic studies.

The rs11003125 polymorphism in the *MBL2* promoter region represents a particularly promising candidate for investigation, given its established functional consequences on gene expression and the relative paucity of data regarding its role in AD across different ethnic groups (22). Understanding the contribution of this genetic variant to AD susceptibility in the Iraqi

population could provide valuable insights into disease mechanisms, identify at-risk individuals, and potentially guide personalised therapeutic approaches.

Therefore, this study was designed to address this knowledge gap by investigating the association between the *MBL2* rs11003125 gene polymorphism, corresponding serum MBL concentrations, and susceptibility to atopic dermatitis in an Iraqi patient cohort. We hypothesised that the C allele of rs11003125 would be associated with reduced serum MBL levels and increased risk of AD, reflecting the importance of intact MBL-mediated innate immunity in protecting against disease development.

Materials and Methods

Study Design and Setting

This case-control study was conducted at Al-Yarmouk Teaching Hospital in Baghdad, Iraq, over four months from early January 2025 to the end of April 2025. The hospital serves as a major referral centre for dermatological conditions in the Baghdad region, providing access to a diverse patient population. The study protocol was designed in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for case-control studies to ensure comprehensive and transparent reporting.

Study Population

Patient Group

The patient group comprised 60 individuals who were clinically diagnosed with atopic dermatitis by consultant dermatologists at the participating hospital. Diagnosis was established based on standardised criteria, including the presence of pruritus, typical morphology and distribution of eczematous lesions, chronic or chronically relapsing course, and personal or family history of atopy. Patients of all ages and both sexes were eligible for inclusion, provided they had an active diagnosis of AD at the time of recruitment. Exclusion criteria for patients included: (1) history of cancer or malignancy; (2) recent chemotherapy or radiation therapy (within the preceding six months); (3) diagnosed immunodeficiency disorders (primary or secondary); (4) autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis); (5) other chronic dermatological conditions (e.g., psoriasis, contact dermatitis) that could confound the diagnosis; (6) other types of eczema (e.g., seborrheic dermatitis, stasis dermatitis); and (7) systemic immunosuppressive therapy within four weeks before sample collection.

Control Group

The control group consisted of 40 healthy individuals who were age- and sex-matched to the patient group at the group level (frequency matching). Controls were recruited from among hospital staff, visitors, and community volunteers who had no personal or family history of atopic dermatitis or other atopic diseases (asthma, allergic rhinitis, food allergy). Additional exclusion criteria for controls were identical to those applied to patients, with the addition of any current or past dermatological condition requiring medical attention. Controls were recruited during the same time period as patients to minimise temporal biases in sample processing and analysis.

Sample Size Considerations

The sample size of 60 patients and 40 controls was determined based on feasibility considerations and previous genetic association studies in similar populations. Given the exploratory nature of this investigation in the Iraqi population, a post-hoc power calculation was performed using the observed allele frequencies. With the minor allele frequency (C allele) of approximately 34% in controls and an odds ratio of 2.18 for AD risk associated with the C allele, the study had approximately 80% power to detect this association at $\alpha = 0.05$ (two-tailed). The sample size is comparable to or exceeds those of previously published studies investigating *MBL2* polymorphisms in various disease contexts.

Blood Sample Collection and Processing

Venous blood samples (5 mL) were collected from each participant under aseptic conditions by trained phlebotomists. Samples were collected into two separate tubes to optimise downstream applications:

Serum separation tube (plain gel tube): Three millilitres of blood were collected in plain gel tubes containing clot activator and gel for serum separation. Tubes were gently inverted 5–6 times and allowed to clot at room temperature for 20–30 minutes. Following clot formation, samples were centrifuged at 3000 rpm for 10 minutes at room temperature. The separated serum was carefully aliquoted into sterile microcentrifuge tubes, labelled with unique study identifiers, and stored at -20°C until analysis of MBL concentrations. Serum samples were protected from repeated freeze-thaw cycles; all aliquots were thawed only once at the time of analysis.

EDTA anticoagulant tube

Two millilitres of blood were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Tubes were gently

inverted several times to ensure thorough mixing and prevent coagulation. Samples were stored at -20°C until DNA extraction, which was performed within two weeks of collection to ensure optimal DNA quality and yield.

Measurement of Serum MBL Concentration

Serum mannose-binding lectin levels were quantified using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit specific for human MBL (Sunlong Biotech, China; catalogue number SL2024Hu). The assay employs the quantitative sandwich enzyme immunoassay technique, utilising a monoclonal antibody specific for human MBL pre-coated onto a microplate.

Assay Principle

Standards, controls, and serum samples were pipetted into the wells, allowing any MBL present to bind to the immobilised antibody. After washing away unbound substances, an enzyme-linked monoclonal antibody specific for MBL was added to the wells. Following a second wash to remove unbound antibody-enzyme reagent, a substrate solution was added, and colour developed in proportion to the amount of MBL bound in the initial step. The colour development was stopped, and the intensity of the colour was measured spectrophotometrically.

Assay Procedure

All reagents, standards, and samples were brought to room temperature (18–25°C) before use. Serum samples were diluted 1:100 with the sample diluent provided in the kit, as recommended by the manufacturer for optimal detection within the standard curve range. Standards were prepared by serial dilution of the provided MBL standard (concentration range: 0–1000 µg/L). Fifty microliters of diluted samples or standards were added to each well in duplicate, and the plate was incubated at 37°C for 30 minutes. After washing five times with wash buffer, 50 µL of horseradish peroxidase (HRP)-conjugated detection antibody was added to each well, followed by incubation at 37°C for 30 minutes. The washing step was repeated, and 50 µL of chromogen solution A and B were added to each well, followed by incubation at 37°C for 15 minutes in the dark. The reaction was stopped by adding 50 µL of stop solution, and the optical density was measured immediately at 450 nm using a microplate reader (BioTek ELx800, USA).

Quality Control

A standard curve was generated by plotting the mean absorbance for each standard against the known concentration, and sample concentrations were interpolated from this curve. The assay

detection range was 5–1000 µg/L, with a sensitivity of 1.0 µg/L. Intra-assay and inter-assay coefficients of variation were <8% and <10%, respectively, as reported by the manufacturer and confirmed in our laboratory through duplicate measurements of randomly selected samples. All samples were analysed in duplicate, and the mean value was used for statistical analysis. Laboratory personnel performing the ELISA were blinded to case-control status to minimise measurement bias.

Genomic DNA Extraction and Quantification

Genomic DNA was extracted from EDTA-anticoagulated whole blood samples using the Presto Mini Blood gDNA Kit (Geneaid Biotech Ltd., Taiwan; catalogue number GB100) according to the manufacturer's protocol. All extraction steps were performed under sterile conditions in a laminar flow hood to prevent contamination.

Extraction Procedure

Briefly, 200 µL of whole blood was mixed with 20 µL of proteinase K and 200 µL of lysis buffer in a 1.5 mL microcentrifuge tube. The mixture was vortexed thoroughly and incubated at 60°C for 10 minutes to ensure complete cell lysis and protein digestion. Following incubation, 200 µL of 96–100% ethanol was added, and the mixture was vortexed again. The entire lysate was transferred to a spin column placed in a collection tube and centrifuged at 14,000–16,000 × g for 2 minutes. The flow-through was discarded, and the column was washed twice with 400 µL of wash buffer (containing ethanol) with centrifugation at 14,000–16,000 × g for 30 seconds each time. The column was dried by additional centrifugation for 3 minutes to remove residual ethanol. Finally, DNA was eluted by adding 100 µL of pre-heated (60°C) elution buffer to the column membrane, incubating for 3–5 minutes, and centrifuging at 6000–8000 × g for 30 seconds.

DNA Quantification and Quality Assessment

The concentration and purity of extracted DNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). DNA concentration was determined by measuring absorbance at 260 nm (A₂₆₀), while purity was evaluated by calculating the A₂₆₀/A₂₈₀ and A₂₆₀/A₂₃₀ ratios. Samples with A₂₆₀/A₂₈₀ ratios between 1.8 and 2.0 and A₂₆₀/A₂₃₀ ratios greater than 1.8 were considered acceptable for downstream applications. DNA integrity was verified by agarose gel electrophoresis (1% agarose) stained with ethidium bromide, visualising intact high-molecular-weight bands without significant

smearing. Extracted DNA was stored at -20°C until PCR amplification.

Primer Design and PCR Amplification of MBL2 rs11003125

Specific oligonucleotide primers were designed to amplify a region of the *MBL2* gene encompassing the rs11003125 polymorphism. Primer design was performed using Primer3 software (version 4.1.0) based on the reference *MBL2* gene sequence obtained from the National Centre for Biotechnology Information (NCBI) database (GenBank accession number NG_008196.1). Primers were designed to have optimal melting temperatures (55–60°C), GC content (40–60%), and minimal self-complementarity or primer-dimer formation potential. The specificity of primer sequences was verified using NCBI BLAST (Basic Local Alignment Search Tool) against the human genome to ensure unique target amplification.

The primer sequences were as follows:

Forward primer: 5'-GCATGCCCTCTGTCCTACAA-3'

Reverse primer: 5'-GGGTTTCCGGATCAAGGGTT-3'

These primers were designed to amplify a 1008-base pair (bp) fragment of the *MBL2* gene containing the rs11003125 polymorphism located in the promoter region. Primers were synthesised commercially (Macrogen Corporation, South Korea) and supplied in lyophilised form. Upon receipt, primers were reconstituted with nuclease-free water to prepare 100 µM stock solutions and stored at -20°C. Working solutions of 10 µM were prepared by

dilution of stock solutions with nuclease-free water.

PCR Reaction Setup: Polymerase chain reaction was performed in a total volume of 25 µL, optimised for efficient and specific amplification. The reaction mixture contained the following components:

12.5 µL of Luna Universal PCR Master Mix (2X) (New England Biolabs, USA; catalogue number M3003) — containing Hot Start Taq DNA polymerase, dNTPs, and optimised buffer components

0.8 µL of forward primer (10 µM) — final concentration 0.32 µM

0.8 µL of reverse primer (10 µM) — final concentration 0.32 µM

2.0 µL of template DNA (approximately 50–100 ng)

8.9 µL of nuclease-free water to achieve the final volume of 25 µL

The Luna Universal PCR Master Mix (2X) contains antibody-mediated hot-start Taq polymerase, which prevents non-specific amplification during reaction setup and the initial denaturation step, thereby enhancing specificity and sensitivity. All PCR setup procedures were performed on ice using sterile, nuclease-free consumables, with appropriate negative controls (no template) included in each run to monitor for contamination.

Thermal Cycling Conditions

Amplification was carried out in a Veriti 96-Well Thermal Cycler (Applied Biosystems, USA) using the following optimised thermal cycling protocol (Table 1):

Table 1: Optimised thermal cycling protocol

Step	Temperature	Duration	Cycles
Initial denaturation	95°C	1 minute	1
Denaturation	95°C	30 seconds	40
Annealing	58°C	30 seconds	40
Extension	68°C	1 minute	40
Final extension	68°C	5 minutes	1
Hold	4°C	∞	1

The annealing temperature of 58°C was determined through gradient PCR optimisation to ensure specific primer binding and minimal non-specific amplification. The extension time of 1 minute at 68°C was sufficient for the complete synthesis of the 1008 bp amplicon, based on the polymerase extension rate of approximately 1 kb/minute.

Gel Electrophoresis and PCR Product Visualisation

Following amplification, PCR products were analysed by agarose gel electrophoresis to

confirm successful amplification and assess product size and specificity.

Gel Preparation: A 2% agarose gel was prepared by dissolving 2 g of molecular biology grade agarose (Invitrogen, USA) in 100 mL of 1× Tris-acetate-EDTA (TAE) buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.0) by heating in a microwave until complete dissolution. The solution was cooled to approximately 50–60°C, and 5 µL of ethidium bromide solution (10 mg/mL) was added to a final concentration of 0.5 µg/mL for DNA visualisation. The gel was poured into a casting tray with appropriate combs and

allowed to solidify at room temperature for 30–40 minutes.

Electrophoresis

Five microliters of each PCR product were mixed with 1 μ L of 6 $\times$ gel loading dye (New England Biolabs, USA) and loaded into the wells. A 100 bp DNA ladder (New England Biolabs, USA; catalogue number N3231) was loaded in the first and last lanes of each gel as a molecular weight marker to enable size estimation of amplified fragments. Electrophoresis was performed in 1 $\times$ TAE buffer at 80 volts and 65 milliamperes (mA) for 60 minutes, or until the dye front had migrated approximately 75–80% of the gel length. Electrophoresis conditions were carefully controlled to ensure optimal separation of DNA fragments.

Visualisation and Documentation

After electrophoresis, DNA bands were visualised under ultraviolet (UV) light using a gel documentation system (Bio-Rad Gel Doc XR+, USA). Images were captured and saved for documentation and analysis. Successful amplification was indicated by the presence of a single, sharp band at the expected size of approximately 1000 bp, as determined by comparison with the DNA ladder. Samples showing successful amplification were selected for sequencing.

Sanger Sequencing and Genotyping

PCR products were purified and sequenced commercially by Macrogen Corporation (South Korea) using Sanger sequencing technology. The purification process removed residual primers, dNTPs, and enzymes that could interfere with sequencing reactions.

Sequencing Procedure

Briefly, PCR products were purified using enzymatic cleanup or column-based methods according to Macrogen's standard protocols. Purified amplicons were subjected to cycle sequencing using BigDye Terminator v3.1 chemistry (Applied Biosystems, USA) on an ABI 3730xl DNA Analyzer (Applied Biosystems, USA). Both forward and reverse strands were sequenced to ensure accurate genotype calls and to resolve any ambiguous positions.

Sequence Analysis

Raw sequencing data (chromatogram files in .ab1 format) were received electronically and analysed using Geneious Prime software (version 2024.0, Biomatters Ltd., New Zealand). The analysis workflow included:

Quality assessment

Chromatograms were visually inspected for baseline noise, signal strength, and peak resolution. Positions with Phred quality scores <20 were flagged for manual review.

Sequence alignment: Forward and reverse sequences for each sample were aligned to generate a consensus sequence, which was then compared to the reference *MBL2* gene sequence using the Geneious Prime alignment tool.

SNP identification

The rs11003125 polymorphism (G/C) was identified by examining the chromatogram at the corresponding position. Genotypes were assigned as follows:

GG genotype: Single G peak at the polymorphic site in both forward and reverse sequences

CC genotype: Single C peak at the polymorphic site in both forward and reverse sequences

GC genotype: Overlapping G and C peaks (double peak) at the polymorphic site, confirmed in both orientations

Quality control

To ensure genotyping accuracy, 20% of randomly selected samples were re-sequenced, and genotype calls were compared with initial results, yielding 100% concordance. Representative chromatograms for each genotype were prepared as supplementary material.

Genotyping success rate was 100%, with all 100 samples (60 patients, 40 controls) successfully sequenced and assigned a genotype. No samples failed during the sequencing process.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism version 9.5.1 (GraphPad Software, USA) and Microsoft Excel 2021 (Microsoft Corporation, USA). The statistical analysis plan was designed to address the study objectives while accounting for the nature of the data and sample size.

Descriptive Statistics

Categorical variables (e.g., sex, genotype distributions) were summarised as frequencies and percentages. Continuous variables were assessed for normality using the Shapiro-Wilk test and visual inspection of histograms and Q-Q plots. Serum MBL concentrations were found to deviate significantly from normal distribution ($p < 0.05$); therefore, these data were expressed as median and interquartile range (IQR: 25th–75th percentile). Age was similarly summarised using median and range due to non-normal distribution.

Comparative Analyses

Demographic comparisons

Differences in age distribution and sex between patients and controls were assessed using the chi-square test for categorical variables and the Mann-Whitney U test for continuous variables.

Genotype and allele frequency analysis

Differences in genotype and allele frequencies between patients and controls were evaluated using the chi-square (χ^2) test or Fisher's exact test (when expected cell counts were <5). Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the strength of association between specific genotypes/alleles and AD susceptibility. The formula for OR calculation was: $OR = (a/b)/(c/d)$, where 'a' is the number of exposed cases, 'b' is the number of unexposed cases, 'c' is the number of exposed controls, and 'd' is the number of unexposed controls.

Hardy-Weinberg equilibrium (HWE)

Deviation from HWE was assessed separately for patients and controls using the chi-square goodness-of-fit test. Expected genotype frequencies under HWE were calculated using the observed allele frequencies: $p^2 + 2pq + q^2 = 1$, where p is the frequency of the G allele, and q is the frequency of the C allele. A p-value < 0.05 was considered indicative of significant deviation from HWE.

Genetic model analysis

Based on the observed genotype distributions and the biological plausibility of a dominant effect of the C allele, a dominant genetic model (GC + CC vs. GG) was analysed to evaluate the effect of carrying at least one C allele on disease susceptibility.

Serum MBL comparisons

Differences in serum MBL levels between patients and controls were analysed using the Mann-Whitney U test (two-group comparison). Differences in serum MBL levels across genotype groups (GG, GC, CC) were analysed using the Kruskal-Wallis test (non-parametric equivalent of one-way ANOVA), followed by post-hoc pairwise comparisons using Dunn's test with Bonferroni correction for multiple testing.

Correlation analysis

The relationship between serum MBL levels and *MBL2* rs11003125 genotypes (coded ordinally as 0 = GG, 1 = GC, 2 = CC) was assessed using Spearman's rank correlation coefficient (ρ), which is appropriate for non-normally distributed data and ordinal variables.

Multivariate logistic regression

To adjust for potential confounding by age and sex, multivariate logistic regression analysis was performed. The dependent variable was AD status (case = 1, control = 0). Independent variables included genotype (dominant model: GC+CC = 1, GG = 0), age (continuous, in years), and sex (female = 1, male = 0). Adjusted odds ratios (AORs) with 95% CIs were calculated, and model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test. Variables were entered into the model simultaneously (enter method) based on biological plausibility and previous literature.

Multiple Comparison Considerations

P-values were not adjusted for multiple comparisons across all analyses, as the study tested a limited number of biologically motivated, pre-specified hypotheses. However, post-hoc pairwise comparisons of MBL levels across three genotype groups were adjusted using Bonferroni correction to control the family-wise error rate. A two-tailed p-value ≤ 0.05 was considered statistically significant for all primary analyses. Exact p-values are reported throughout to allow readers to assess the strength of evidence.

Results

Demographic Characteristics of the Study Population

The study included 100 participants, comprising 60 patients with atopic dermatitis and 40 age- and sex-matched healthy controls. The demographic characteristics of both groups were analysed to ensure comparability and to identify any potential confounders requiring adjustment in subsequent analyses.

Age Distribution: The age of participants ranged from 1 to 82 years across both groups. Among patients with AD, the highest proportion was observed in the elderly group (>60 years), accounting for 18 individuals (30.0% of the patient cohort). This was followed by middle-aged adults (30–59 years) comprising 15 patients (25.0%), young adults (18–29 years) with 13 patients (21.7%), children aged 6–11 years (late childhood) with 6 patients (10.0%), adolescents (12–17 years) with 5 patients (8.3%), and early childhood (0–5 years) with 3 patients (5.0%). The control group showed a broadly similar age distribution, with the largest proportion also in the elderly group (14 individuals, 35.0%), followed by middle-aged adults (11 individuals, 27.5%), young adults (6 individuals, 15.0%), adolescents (4 individuals, 10.0%), late childhood (3 individuals, 7.5%), and early childhood (2 individuals, 5.0%). Statistical analysis confirmed no significant difference in age distribution between patients and controls ($\chi^2 = 1.82$, $df = 5$, $p = 0.87$), indicating successful

frequency matching. Table 2 presents the detailed age distribution across both groups.

Table 2: Distribution of Patients and Controls According to Age Groups

Age Group (Years)	Life Stage	Patients (n=60) No. (%)	Controls (n=40) No. (%)	Total No. (%)
0–5	Early childhood	3 (5.0)	2 (5.0)	5 (5.0)
6–11	Late childhood	6 (10.0)	3 (7.5)	9 (9.0)
12–17	Adolescence	5 (8.3)	4 (10.0)	9 (9.0)
18–29	Young adult	13 (21.7)	6 (15.0)	19 (19.0)
30–59	Middle adult	15 (25.0)	11 (27.5)	26 (26.0)
>60	Elderly	18 (30.0)	14 (35.0)	32 (32.0)
Total		60 (100.0)	40 (100.0)	100 (100.0)

* $\chi^2 = 1.82$, df = 5, p = 0.87 (non-significant)*

Sex Distribution

The patient group comprised 36 females (60.0%) and 24 males (40.0%), yielding a female-to-male ratio of 1.5:1. The control group included 26 females (65.0%) and 14 males (35.0%), with a female-to-male ratio of 1.86:1. Chi-square

analysis revealed no statistically significant difference in sex distribution between patients and controls ($\chi^2 = 0.26$, df = 1, p = 0.61), confirming adequate matching on this demographic variable. Table 3 summarises the sex distribution.

Table 3: Distribution of Patients and Controls According to Sex

Sex	Patients (n=60) No. (%)	Controls (n=40) No. (%)	Total No. (%)
Female	36 (60.0)	26 (65.0)	62 (62.0)
Male	24 (40.0)	14 (35.0)	38 (38.0)
Total	60 (100.0)	40 (100.0)	100 (100.0)

* $\chi^2 = 0.26$, df = 1, p = 0.61 (non-significant)*

Clinical Characteristics of Atopic Dermatitis Patients

Among the 60 patients with AD, disease severity was categorised based on clinical presentation as either acute or chronic, and the relationship between disease duration and severity was examined. Acute cases were defined by recent onset of symptoms with active inflammatory lesions, while chronic cases were characterised by persistent or recurrent disease with lichenification and chronic skin changes.

The analysis revealed a highly significant association between disease duration and severity (p < 0.0001). Among patients with a disease duration of less than one month, the

majority (11 patients, 57.9%) presented with acute manifestations, while none had chronic disease. In contrast, patients with longer disease duration predominantly exhibited chronic features. Among those with disease duration of 1–6 months, 12 patients (29.3% of chronic cases) had chronic AD, while 8 patients (42.1% of acute cases) still presented with acute manifestations. All patients with disease duration exceeding six months—including those with 6 months to one year (11 patients, 26.8% of chronic cases), 1–5 years (9 patients, 22.0% of chronic cases), and greater than five years (9 patients, 22.0% of chronic cases)—had chronic AD. Table 4 presents these findings in detail.

Table 4: Distribution of Atopic Dermatitis Severity According to Disease Duration

Disease Duration	Acute (n=19) No. (%)	Chronic (n=41) No. (%)	Total (n=60) No. (%)
< 1 month	11 (57.9)	0 (0.0)	11 (18.3)
1–6 months	8 (42.1)	12 (29.3)	20 (33.3)
6 months – 1 year	0 (0.0)	11 (26.8)	11 (18.3)
1–5 years	0 (0.0)	9 (22.0)	9 (15.0)
> 5 years	0 (0.0)	9 (22.0)	9 (15.0)
Total	19 (100.0)	41 (100.0)	60 (100.0)

* $\chi^2 = 37.84$, df = 4, p < 0.0001 (highly significant)*

DNA Extraction and PCR Amplification of MBL2 Gene Fragment

Genomic DNA was successfully extracted from all 100 blood samples (60 patients, 40 controls)

using the Presto Mini Blood gDNA Kit. Spectrophotometric analysis revealed mean DNA concentrations of 45.6 ± 15.2 ng/ μ L (range: 20.1–89.3 ng/ μ L) with A260/A280 ratios between 1.82 and 1.96, indicating high purity suitable for PCR amplification.

PCR amplification of the *MBL2* gene fragment encompassing the rs11003125 polymorphism yielded a single, distinct band of approximately 1000 bp in all samples, as visualised by agarose

gel electrophoresis. Figure 1 presents representative gel electrophoresis results for control samples, while Figure 2 shows results for patient samples. The 100 bp DNA ladder (lane M) enabled size verification, with the target amplicon migrating between the 900 bp and 1100 bp markers, consistent with the expected 1008 bp fragment. The absence of non-specific bands or primer-dimers indicated high specificity of the optimised PCR conditions.

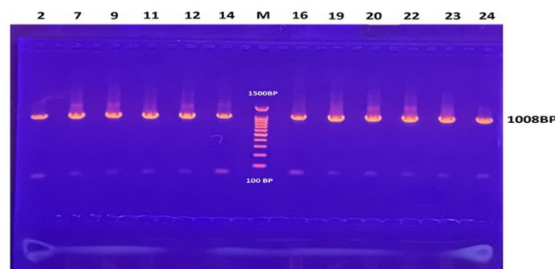


Figure 1: Agarose Gel Electrophoresis of *MBL2* Gene Amplification Products (Control Samples)

PCR products from control samples were electrophoresed on 2% agarose gel at 80 volts and 65 mA for 60 minutes and visualised under UV light after ethidium bromide staining. Lane M: 100 bp DNA ladder; lanes 1–8: control samples showing single bands at approximately 1000 bp, confirming successful amplification of the target *MBL2* gene fragment

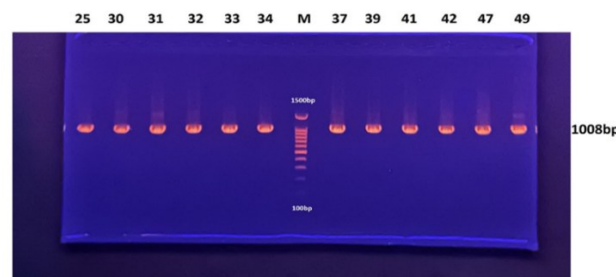


Figure 2: Agarose Gel Electrophoresis of *MBL2* Gene Amplification Products (Patient Samples)

PCR products from patient samples were electrophoresed on 2% agarose gel at 80 volts and 65 mA for 60 minutes and visualised under UV light after ethidium bromide staining. Lane M: 100 bp DNA ladder; lanes 1–8: patient samples showing single bands at approximately 1000 bp, confirming successful amplification of the target *MBL2* gene fragment

Sequencing and Genotyping of *MBL2* rs11003125 Polymorphism

Sanger sequencing of purified PCR products from all 100 samples was completed, with high-quality chromatograms obtained for each sample. Visual inspection of electropherograms using Geneious Prime software enabled clear discrimination of the three possible genotypes at the rs11003125 polymorphic site.

Figure 3 illustrates representative electropherograms for each genotype.

GG genotype: Characterised by a single, sharp G peak at the polymorphic position in both forward and reverse sequences

CC genotype: Characterised by a single, sharp C peak at the polymorphic position in both forward and reverse sequences

GC genotype: Characterised by overlapping G and C peaks (double peak) at the polymorphic position, confirmed in both sequencing orientations

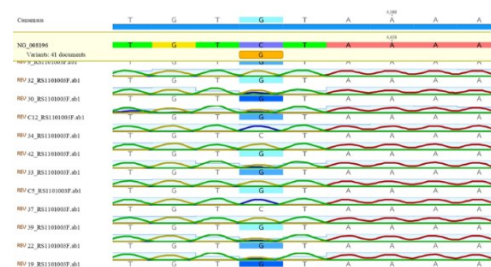


Figure 3: Representative Electropherograms of MBL2 rs11003125 Genotypes

Sanger sequencing chromatograms showing the three genotypes of the MBL2 rs11003125 polymorphism. (A) GG genotype: single G peak; (B) GC genotype: overlapping G and C peaks; (C) CC genotype: single C peak. The polymorphic site is indicated by arrows

Genotype and Allele Frequency Distribution

Analysis of genotype and allele frequencies revealed significant differences between AD patients and healthy controls, as shown in Table 5.

Genotype Distribution

Among the 60 AD patients, the GC heterozygous genotype was most frequent, observed in 29 individuals (48.33%), followed by the CC homozygous mutant genotype in 18 individuals (30.00%), and the GG homozygous wild-type genotype in 13 individuals (21.67%). In contrast, among the 40 controls, the GG genotype was predominant, found in 21 individuals (52.50%), followed by GC in 11 individuals (27.50%) and CC in 8 individuals (20.00%). The overall difference in genotype distribution between patients and controls was statistically significant ($\chi^2 = 9.95$, $df = 2$, $p = 0.007$).

Risk Association by Genotype

Using the GG genotype as the reference category, both the GC and CC genotypes were associated with significantly increased risk of AD.

Individuals with the GC genotype had an odds ratio of 4.26 (95% CI: 1.65–10.98, $p = 0.002$), indicating a greater than four-fold increase in AD risk compared to GG homozygotes. Those with the CC genotype showed an odds ratio of 3.63 (95% CI: 1.23–10.67, $p = 0.019$), representing a nearly four-fold increase in risk. These findings suggest that carriage of the C allele, whether in a heterozygous or homozygous state, confers substantial susceptibility to AD.

Allele Frequency Distribution

Analysis of allele frequencies revealed that the C allele was significantly more common in patients than in controls. Among patients, the C allele frequency was 52.6% (61 of 116 alleles), while among controls it was 33.75% (27 of 80 alleles). The G allele showed the opposite pattern, with frequencies of 47.41% in patients and 66.25% in controls. The association between the C allele and AD risk was highly significant (OR = 2.18, 95% CI: 1.22–3.90, $p = 0.006$), confirming that carriage of the C allele is a significant risk factor for AD in this population.

Table 5: Genotype Distribution and Allele Frequency of MBL2 rs11003125 in Patients and Controls

Genotype/Allele	Patients (n=60) No. (%)	Controls (n=40) No. (%)	OR (95% CI)	p-value
Genotype				
GG	13 (21.67)	21 (52.50)	Reference	—
GC	29 (48.33)	11 (27.50)	4.26 (1.65–10.98)	0.002
CC	18 (30.00)	8 (20.00)	3.63 (1.23–10.67)	0.019
* $\chi^2 = 9.95$, $df = 2$, $p = 0.007$ *				
Allele				
G	55 (47.41)	53 (66.25)	Reference	—
C	61 (52.59)	27 (33.75)	2.18 (1.22–3.90)	0.006
* $\chi^2 = 6.76$, $df = 1$, $p = 0.009$ *				

Dominant Genetic Model Analysis

Based on the observation that both GC and CC genotypes were associated with increased AD risk, a dominant genetic model was constructed comparing individuals carrying at least one C allele (GC + CC) against GG homozygotes. This

model tests the hypothesis that the presence of the C allele, regardless of zygosity, confers increased disease susceptibility.

As shown in Table 6, among patients, 47 individuals (78.33%) carried at least one C allele (29 GC + 18 CC), compared to only 13 (21.67%)

with the GG genotype. Among controls, 19 individuals (47.50%) carried at least one C allele (11 GC + 8 CC), while 21 (52.50%) had the GG genotype. The dominant model analysis revealed a highly significant association between C allele

carriage and AD risk (OR = 3.99, 95% CI: 1.68–9.49, $p = 0.003$), indicating that individuals carrying at least one C allele have approximately four-fold higher odds of having AD compared to GG homozygotes.

Table 6: Dominant Model Analysis of MBL2 rs11003125 Polymorphism and AD Risk

Genotype Comparison	Patients (n=60) No. (%)	Controls (n=40) No. (%)	OR (95% CI)	p-value
GG	13 (21.67)	21 (52.50)	Reference	—
GC + CC	47 (78.33)	19 (47.50)	3.99 (1.68–9.49)	0.003

Multivariate Logistic Regression Analysis

To account for potential confounding by demographic variables, multivariate logistic regression analysis was performed, including genotype (dominant model: GC+CC vs. GG), age (continuous, years), and sex (female vs. male) as independent variables, with AD status as the dependent variable. The results are presented in Table 7.

After adjustment for age and sex, the association between C allele carriage and AD risk remained highly significant. Individuals carrying at least one C allele had an adjusted odds ratio (AOR) of 3.90

(95% CI: 1.54–9.24, $p = 0.004$) compared to GG homozygotes, virtually unchanged from the crude OR of 3.99. This indicates that the observed association is robust and independent of age and sex effects.

Neither age (AOR = 1.01, 95% CI: 0.98–1.03, $p = 0.52$) nor sex (AOR = 1.12, 95% CI: 0.56–2.21, $p = 0.74$) showed significant independent associations with AD risk in this model. The Hosmer-Lemeshow goodness-of-fit test indicated adequate model fit ($\chi^2 = 5.84$, $df = 8$, $p = 0.67$), supporting the validity of the regression analysis.

Table 7: Multivariate Logistic Regression Analysis of Factors Associated with AD Risk

Variable	Adjusted OR	95% CI	p-value
GC+CC vs. GG	3.9	1.54–9.24	0.004
Age (years)	1.01	0.98–1.03	0.52
Sex (female vs. male)	1.12	0.56–2.21	0.74

Hardy-Weinberg Equilibrium Analysis

Hardy-Weinberg equilibrium (HWE) was assessed separately for patients and controls to evaluate whether genotype frequencies conformed to population expectations and to detect potential genotyping errors or population stratification (Table 8).

In the control group, observed genotype frequencies were consistent with HWE expectations. The expected frequencies based on allele counts (G allele frequency = 0.6625, C allele frequency = 0.3375) were: GG = 21.6, GC = 11.2, CC = 7.2. The observed frequencies (GG = 21, GC = 11, CC = 8) showed minimal deviation

($\chi^2 = 2.91$, $df = 1$, $p = 0.065$), indicating that the control population was in HWE.

In contrast, the patient group showed significant deviation from HWE. Based on allele frequencies (G = 0.4741, C = 0.5259), expected genotype frequencies were: GG = 10.5, GC = 39.4, CC = 10.1. The observed frequencies (GG = 13, GC = 29, CC = 18) deviated significantly from these expectations ($\chi^2 = 4.04$, $df = 1$, $p = 0.03$). This deviation from HWE in the patient group is not unexpected in case-control studies of genetic associations, as it may reflect the true biological association between the polymorphism and disease susceptibility (i.e., the polymorphism is not neutral with respect to disease risk).

Table 8: Hardy-Weinberg Equilibrium Analysis of MBL2 rs11003125 Genotypes

Group	Genotype	Observed (n)	Expected (n)	χ^2	p-value
Patients	GG	13	10.5	4.04	0.03
	GC	29	39.4		
	CC	18	10.1		
Controls	GG	21	21.6	2.91	0.065
	GC	11	11.2		
	CC	8	7.2		

Serum MBL Levels: Patients vs. Controls

Serum MBL concentrations were measured in all 100 participants using ELISA. Due to the non-

normal distribution of MBL levels (Shapiro-Wilk test: $p < 0.001$), non-parametric statistical methods were employed for all analyses involving this variable.

As shown in Table 9, AD patients had significantly lower serum MBL levels compared to healthy controls. The median serum MBL concentration in patients was 84.42 $\mu\text{g/L}$ (IQR: 42.59–139.79 $\mu\text{g/L}$), whereas in controls it was substantially higher at 147.62 $\mu\text{g/L}$ (IQR: 49.65–

526.49 $\mu\text{g/L}$). The Mann-Whitney U test confirmed that this difference was statistically significant ($U = 805.0$, $Z = -2.779$, $p = 0.005$).

The wide interquartile range in the control group reflects considerable inter-individual variability in MBL levels, consistent with the known population variation influenced by genetic polymorphisms. Despite this variability, the central tendency and distribution clearly differentiate patients from controls.

Table 9: Comparison of Serum MBL Levels Between AD Patients and Controls

Group	N	Median MBL ($\mu\text{g/L}$)	IQR ($\mu\text{g/L}$)	U-value	Z-score	p-value
Patients	60	84.42	42.59–139.79	805	-2.779	0.005
Controls	40	147.62	49.65–526.49			

Mann-Whitney U test

Relationship Between MBL2 Genotype and Serum MBL Levels in Patients

A key finding of this study was the strong genotype-dependent variation in serum MBL levels among AD patients, as presented in Table 10.

Patients with the GG genotype had the highest median MBL concentration at 133.49 $\mu\text{g/L}$ (IQR: 53.12–157.01 $\mu\text{g/L}$). Those with the GC

genotype showed intermediate levels, with a median of 87.58 $\mu\text{g/L}$ (IQR: 61.38–142.48 $\mu\text{g/L}$). Patients with the CC genotype had the lowest levels, with a median of only 36.68 $\mu\text{g/L}$ (IQR: 21.82–46.57 $\mu\text{g/L}$). The Kruskal-Wallis test revealed highly significant differences among the three genotype groups ($H = 15.84$, $df = 2$, $p = 0.0004$).

Table 10: Relationship Between MBL2 rs11003125 Genotype and Serum MBL Levels in AD Patients

Genotype	N	Median MBL ($\mu\text{g/L}$)	IQR ($\mu\text{g/L}$)	p-value
GG	13	133.49	53.12–157.01	0.0004
GC	29	87.58	61.38–142.48	
CC	18	36.68	21.82–46.57	

Kruskal-Wallis test

To further characterise the differences among genotypes, post-hoc pairwise comparisons were performed using Dunn's test with Bonferroni correction for multiple testing (Table 11). All pairwise comparisons were statistically significant after adjustment:

GG vs. GC: Mean rank difference = 45.91, adjusted $p = 0.015$, indicating significantly higher MBL levels in GG compared to GC genotype carriers.

GG vs. CC: Mean rank difference = 96.81, adjusted $p < 0.001$, demonstrating markedly

higher MBL levels in GG compared to CC genotype carriers.

GC vs. CC: Mean rank difference = 50.90, adjusted $p = 0.002$, confirming significantly higher MBL levels in GC compared to CC genotype carriers.

These results demonstrate a clear gene-dosage effect, where each C allele is associated with progressively lower serum MBL concentrations, consistent with the known functional impact of this promoter polymorphism on gene expression.

Table 11: Post-hoc Pairwise Comparisons of Serum MBL Levels by Genotype

Genotype Comparison	Mean Rank Difference	Adjusted p-value	Significance
GG vs. GC	45.91	0.015	Significant
GG vs. CC	96.81	<0.001	Highly significant
GC vs. CC	50.9	0.002	Significant

Dunn's test with Bonferroni correction for multiple comparisons

Correlation Between MBL2 Genotype and Serum MBL Levels

Spearman's rank correlation analysis was performed to quantify the relationship between MBL2 rs11003125 genotype (coded ordinaly: 0

= GG, 1 = GC, 2 = CC) and serum MBL concentrations in AD patients (Table 12). The analysis revealed a significant negative correlation between genotype and MBL levels ($\rho = -0.462$, $p = 0.0002$), indicating that as the number of C alleles increases, serum MBL

concentrations decrease. The magnitude of the correlation coefficient ($\rho = -0.462$) suggests a moderate-to-strong inverse relationship, with genotype accounting for approximately 21% of the variance in MBL levels (coefficient of determination $r^2 = 0.21$).

Table 12: Correlation Between MBL2 Genotype and Serum MBL Levels

Parameters	N	Spearman's ρ	p-value
MBL serum levels vs. MBL2 rs11003125 genotype	60	-0.462	0.0002

Discussion

This case-control study provides compelling evidence for a significant association between the *MBL2* rs11003125 promoter polymorphism, reduced serum MBL concentrations, and increased susceptibility to atopic dermatitis in an Iraqi population. Our findings demonstrate that the C allele of rs11003125 is associated with a greater than two-fold increased risk of AD at the allelic level, and individuals carrying at least one C allele have nearly four-fold higher odds of disease compared to GG homozygotes. Furthermore, we observed a clear genotype-dependent gradient in serum MBL levels among patients, with CC homozygotes exhibiting the lowest concentrations, GG homozygotes the highest, and heterozygotes showing intermediate levels. These results align with the established functional role of this polymorphism in regulating *MBL2* gene expression and underscore the importance of intact MBL-mediated innate immunity in protecting against AD development.

Demographic and Clinical Characteristics

The demographic profile of our patient cohort revealed several noteworthy features. The higher proportion of female patients (60%) compared to males (40%), while not statistically significant compared to controls, is consistent with the female predominance reported in adult AD populations (23). This pattern has been attributed to hormonal influences on immune function and skin barrier integrity, with oestrogen and progesterone modulating various aspects of cutaneous biology and inflammatory responses (24). The absence of significant sex differences between patients and controls in our study confirms successful matching and eliminates sex as a major confounding factor in the genetic association analyses.

The age distribution of our patient population, with the largest proportion (30%) in the elderly group (>60 years), differs from the classical understanding of AD as predominantly a childhood disease. However, this finding aligns with growing recognition that AD has a substantial burden in older adults (25). Population-based studies have demonstrated

that AD can persist into older age or have late-onset presentations, with prevalence estimates of 1–3% in elderly populations (26). The hospital-based recruitment in our study may have enriched for patients with chronic, persistent disease requiring specialist care, potentially explaining the higher representation of older adults. Additionally, the variable age distribution may reflect the complex natural history of AD, which can follow different trajectories, including early-onset persistent, early-onset resolving, and late-onset patterns (27).

The strong association between disease duration and chronicity observed in our patients ($p < 0.0001$) is clinically expected and validates the accuracy of our clinical characterisation. The finding that all patients with disease duration exceeding six months had chronic manifestations aligns with the natural progression of AD, where acute flares in the context of chronic disease are common, but purely acute presentations become less frequent as disease duration extends (28).

MBL2 rs11003125 Polymorphism and AD Susceptibility

The central finding of this study, the significant association between the *MBL2* rs11003125 polymorphism and AD risk, adds to the growing body of evidence implicating innate immune genes in AD pathogenesis. The odds ratios of 4.26 for GC heterozygotes and 3.63 for CC homozygotes represent substantial effect sizes, suggesting that this polymorphism is a major genetic determinant of AD susceptibility in the Iraqi population. The dominant model analysis, with an OR of 3.99 for C allele carriers, indicates that even heterozygous carriage confers significantly increased risk, highlighting the biological importance of maintaining adequate MBL levels.

Our findings are consistent with those of Kim et al. (18), who reported associations between specific *MBL2* haplotypes and AD in a Korean population. However, direct comparison is complicated by differences in the polymorphisms studied and haplotype structures across populations. The rs11003125 polymorphism, located in the promoter region at position -550, is part of a complex haplotype that includes other

functional variants in the promoter (rs11003124 at -221) and exon 1 (rs1800450, rs1800451, rs5030737) (29). The functional consequences of these variants are interdependent, with certain haplotypes (termed "low-producer" or "deficient" haplotypes) associated with markedly reduced MBL levels (30). Our finding that rs11003125 alone shows a strong association with both MBL levels and AD risk suggests that this polymorphism either has independent functional effects or is in strong linkage disequilibrium with other causal variants in the Iraqi population. The deviation from Hardy-Weinberg equilibrium observed in our patient group ($p = 0.03$), but not in controls ($p = 0.065$), warrants careful consideration. In case-control genetic association studies, deviation from HWE in cases but not controls is often interpreted as evidence for a true association between the polymorphism and disease (31). This pattern arises because the polymorphism is not neutral with respect to disease risk, leading to genotype frequencies that differ from population expectations. The alternative explanation—genotyping error—is less likely given the high-quality sequencing data, 100% genotyping success rate, and confirmation through duplicate sequencing of randomly selected samples. Furthermore, the genotype-dependent gradient in MBL levels provides biological validation that our genotyping accurately reflects functional variation.

Genotype-Dependent Variation in Serum MBL Levels

The strong association between *MBL2* rs11003125 genotype and serum MBL concentrations in our patients provides crucial functional validation of the genetic findings. The clear gene-dosage effect—with CC homozygotes having the lowest levels, GG homozygotes the highest, and heterozygotes intermediate—is precisely what would be expected for a promoter polymorphism affecting transcription efficiency (32). The magnitude of difference is clinically meaningful: median MBL levels in CC homozygotes (36.68 µg/L) were less than one-third of those in GG homozygotes (133.49 µg/L) and less than half of those in heterozygotes (87.58 µg/L).

These findings align with previous functional studies of the rs11003125 polymorphism. The variant, also known as the H/L variant based on historical nomenclature, affects the binding of transcription factors to the *MBL2* promoter (33). The C allele creates a binding site for a repressor protein or disrupts a binding site for an activator, resulting in reduced transcriptional activity (34). In vitro studies using reporter gene constructs have demonstrated that promoter haplotypes containing the C allele have 2- to 5-fold lower

activity compared to those with the G allele (35). Our in vivo findings in AD patients are consistent with these experimental observations.

The significant negative correlation between C allele count and MBL levels ($\rho = -0.462$, $p = 0.0002$) further strengthens this relationship. The moderate-to-strong correlation coefficient indicates that genotype is a major determinant of MBL variation in our patient population, although other factors—including inflammation, liver function, and additional genetic variants—likely contribute to the remaining variance (36).

Biological Plausibility and Mechanistic Implications

The association between MBL deficiency and AD susceptibility is biologically plausible through multiple interconnected mechanisms involving host defence, immune regulation, and barrier function.

Impaired Antimicrobial Defence: *Staphylococcus aureus* colonises the skin of up to 90% of AD patients, compared to approximately 20% of healthy individuals, and plays a central role in disease pathogenesis through superantigen production, protease secretion, and direct barrier disruption (37). MBL binds to carbohydrate structures on the *S. aureus* surface, including teichoic acids and peptidoglycan components, facilitating opsonisation and phagocytosis by neutrophils and macrophages (38). MBL deficiency may therefore impair clearance of *S. aureus*, allowing bacterial proliferation and perpetuating the cycle of inflammation and barrier damage characteristic of AD. A recent study by Abood (39) documented high rates of *S. aureus* colonisation in Iraqi AD patients, underscoring the clinical relevance of this mechanism in our population.

Complement Pathway Dysregulation

MBL initiates the lectin pathway of complement activation upon binding to microbial surfaces, leading to the formation of the membrane attack complex and direct microbial killing (40). Beyond its antimicrobial effects, complement activation generates anaphylatoxins (C3a, C5a) that recruit and activate immune cells, influencing the character of the adaptive immune response (41). Recent work has highlighted the importance of complement in shaping Th2-polarised immunity at barrier surfaces, with complement components promoting Th2 differentiation and enhancing allergic inflammation (16). MBL deficiency may alter the quality or magnitude of complement activation at the skin surface, potentially skewing immune responses toward pathogenic Th2 patterns.

Microbial Sensitisation and IgE Responses

Belfrage et al. (14) recently reported that MBL deficiency was significantly associated with increased IgE sensitisation to *Candida albicans* in AD patients, and low MBL levels correlated with more severe disease. This finding suggests that impaired MBL-mediated recognition of fungal and bacterial antigens may lead to aberrant adaptive immune responses and heightened IgE production. Given the role of IgE in mediating immediate hypersensitivity reactions and contributing to chronic allergic inflammation, this mechanism may be particularly relevant to AD pathogenesis.

Apoptotic Cell Clearance

MBL also functions as a pattern recognition molecule for apoptotic cells, binding to surface structures exposed during programmed cell death and facilitating their clearance by phagocytes (42). Efficient clearance of apoptotic cells is essential for resolving inflammation and preventing autoimmune responses. In the chronically inflamed skin of AD patients, impaired clearance of apoptotic keratinocytes and infiltrating inflammatory cells may perpetuate inflammation and delay healing. MBL deficiency could therefore contribute to the chronicity and persistence of AD lesions.

Interaction with Skin Barrier Function

The most well-established genetic risk factor for AD is loss-of-function mutations in the filaggrin gene (*FLG*), which encodes a protein critical for epidermal barrier integrity (43). Filaggrin deficiency leads to increased transepidermal water loss, enhanced allergen penetration, and altered skin microbiome composition (44). It is plausible that defects in both the physical barrier (due to *FLG* mutations) and the innate immune barrier (due to *MBL2* polymorphisms) could have synergistic effects, compounding disease risk and severity. Future studies examining gene-gene interactions between *MBL2* and *FLG* variants would be valuable.

Comparison with Other Populations and Studies

Our findings contribute to the global understanding of *MBL2* genetic variation and its role in AD across different ethnic groups. The C allele frequency in our Iraqi control population (33.75%) is similar to frequencies reported in other Middle Eastern populations but differs from European and Asian populations (45). This highlights the importance of population-specific studies, as genetic associations may vary depending on the underlying haplotype structure and allele frequencies.

In a Korean study, Kim et al. (18) examined multiple *MBL2* polymorphisms and haplotypes in

AD patients, finding associations with specific low-producing haplotypes. However, the specific polymorphisms contributing to these associations differed somewhat from our findings, reflecting population differences in linkage disequilibrium patterns. Studies in European populations have yielded inconsistent results, with some reporting associations between *MBL2* variants and atopic phenotypes while others have not (46). These discrepancies may arise from differences in study design, sample size, population genetic background, and environmental exposures that modify the effect of genetic variants.

The role of MBL in other inflammatory and autoimmune diseases has been extensively studied. Associations have been reported between *MBL2* polymorphisms and systemic lupus erythematosus, rheumatoid arthritis, and various infectious diseases, although effect sizes vary across studies (47). The consistent theme emerging from this literature is that MBL deficiency is generally associated with increased susceptibility to infection and, in some contexts, with altered risk of autoimmune and inflammatory conditions. Our findings extend this paradigm to atopic dermatitis, reinforcing the concept that intact MBL-mediated innate immunity is important for maintaining immune homeostasis at barrier surfaces.

Study Limitations

This study has several limitations that should be considered when interpreting the findings.

Sample Size

The relatively modest sample size (60 patients, 40 controls) limits the statistical power for detecting small effect sizes and precludes detailed subgroup analyses based on disease severity, age of onset, or other clinical characteristics. While our post-hoc power calculation indicated adequate power to detect the observed effect size for the primary association, replication in larger independent cohorts is needed to confirm these findings and obtain more precise effect estimates.

Single-Centre Design

Recruitment from a single hospital in Baghdad may introduce selection bias, and the findings may not be generalizable to all Iraqi AD patients or to other populations. Geographic and ethnic diversity within Iraq could influence both allele frequencies and disease characteristics, and multicentre studies would be valuable.

Population Stratification

Although we matched patients and controls for age and sex, we did not comprehensively assess

ancestry or employ genomic control methods to detect and adjust for population stratification. Unmeasured genetic differences between groups could potentially confound association results, although the consistency of our findings with functional studies of the polymorphism mitigates this concern.

Limited Genotyping

We examined only one *MBL2* polymorphism (rs11003125) and did not assess other functional variants in the promoter (e.g., rs11003124 at -221) or exon 1 (e.g., rs1800450, rs1800451, rs5030737) that are known to influence MBL levels. Comprehensive haplotype analysis would provide a more complete picture of *MBL2* variation and its relationship to AD risk in this population.

Cross-Sectional Design

The case-control design precludes the determination of the temporal relationship between MBL deficiency and AD development. While our findings are consistent with MBL deficiency predisposing to AD, we cannot exclude the possibility that AD or its treatment influences MBL levels. However, the genetic basis of MBL variation makes reverse causation unlikely.

Lack of Functional Assessment

We measured MBL protein concentrations but did not assess functional activity of the lectin pathway (e.g., MBL-mediated complement deposition). Some studies suggest that functional assays may provide additional information beyond protein levels (48).

No Assessment of Other Atopic Phenotypes

We did not collect comprehensive data on other atopic conditions (asthma, allergic rhinitis, food allergy) in patients or their family members, which could provide additional insights into the role of MBL in the broader atopic diathesis.

Disease Severity Assessment

While we categorised disease as acute or chronic based on duration and clinical presentation, we did not employ validated severity scoring systems such as the SCORAD (SCORing Atopic Dermatitis) index or EASI (Eczema Area and Severity Index), which would have enabled more detailed genotype-phenotype correlations.

Future Directions

These limitations point to several important directions for future research.

Replication and Extension

Larger multicentre studies across diverse Iraqi populations are needed to replicate and extend our findings. Such studies should include comprehensive genotyping of all known functional *MBL2* variants, haplotype analysis, and assessment of gene-gene interactions with other AD risk loci (e.g., *FLG*, *TSLP*, *IL4R*).

Functional Studies

Mechanistic studies using in vitro models and patient-derived samples could elucidate the precise molecular mechanisms by which rs11003125 and related variants influence *MBL2* transcription and MBL production. Assessment of MBL functional activity (complement deposition assays) in relation to genotype and disease status would provide additional insights.

Longitudinal Studies

Prospective cohort studies following individuals from birth or early childhood could determine whether MBL deficiency precedes AD development and whether it influences disease course, severity, and complications such as infections.

Skin-Level Investigations

Measurement of MBL levels and activity in the skin (rather than serum) and assessment of MBL deposition in skin lesions could provide more direct evidence for local functional effects. Studies examining the interaction between MBL, skin microbiome composition, and antimicrobial peptide expression would be valuable.

Therapeutic Implications

If confirmed in larger studies, the association between MBL deficiency and AD risk raises the question of whether MBL replacement or augmentation could have therapeutic potential. Recombinant MBL has been developed and studied in clinical trials for MBL-deficient patients with infections (49), but its utility in AD has not been explored.

Gene-Environment Interactions

Investigation of interactions between *MBL2* genotype and environmental factors such as early-life infections, antibiotic exposure, and dietary factors could reveal modifiable risk factors that interact with genetic susceptibility.

Conclusion

This study provides strong evidence for an association between the *MBL2* rs11003125 gene polymorphism, reduced serum MBL concentrations, and increased risk of atopic dermatitis in the Iraqi population. The C allele of

rs11003125 was significantly more frequent in AD patients than in controls and was associated with a clear genotype-dependent reduction in serum MBL levels, with CC homozygotes exhibiting the lowest concentrations. Individuals carrying at least one C allele had nearly fourfold higher odds of AD compared to GG homozygotes, and this association remained significant after adjustment for age and sex. These findings highlight the critical role of MBL-mediated innate immunity in AD pathogenesis and add to the growing recognition that genetic variation in innate immune genes contributes to susceptibility to allergic diseases. The results underscore the importance of considering population-specific genetic factors in understanding disease mechanisms and may ultimately inform personalised approaches to risk prediction and therapeutic intervention. Further research with larger, more diverse cohorts and comprehensive functional assessment is needed to validate these findings and explore their clinical implications.

List of Abbreviations

AD: Atopic Dermatitis
 AOR: Adjusted Odds Ratio
 BLAST: Basic Local Alignment Search Tool
 Bp: Base pair
 CI: Confidence Interval
 DNA: Deoxyribonucleic Acid
 dNTP: Deoxynucleotide Triphosphate
 EASI: Eczema Area and Severity Index
 EDTA: Ethylenediaminetetraacetic Acid
 ELISA: Enzyme-Linked Immunosorbent Assay
 FLG: Filaggrin Gene
 HWE: Hardy-Weinberg Equilibrium
 HRP: Horseradish Peroxidase
 IgE: Immunoglobulin E
 IQR: Interquartile Range
 IRB: Institutional Review Board
 MBL: Mannose-Binding Lectin
 MBL2: Mannose-Binding Lectin 2 Gene
 NCBI: National Centre for Biotechnology Information
 OR: Odds Ratio
 PCR: Polymerase Chain Reaction
 Rpm: Revolutions Per Minute
 SCORAD: SCORing Atopic Dermatitis
 SNP: Single Nucleotide Polymorphism
 STROBE: Strengthening the Reporting of Observational Studies in Epidemiology
 TAE: Tris-Acetate-EDTA
 Th2: T-helper 2
 UV: Ultraviolet

Declarations

Ethics Approval and Consent to Participate

This study was conducted in accordance with the ethical standards of the institutional and national

research committees and with the 1964 Helsinki declaration and its later amendments. The study protocol was approved by the Ethics Committee of the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, and the Iraqi Ministry of Health Ethics Committee (Approval Number: 312; Date: January 20, 2025). Written informed consent was obtained from all individual participants included in the study. For participants under the age of 18 years, written informed consent was obtained from a parent or legal guardian. All participants were informed about the purpose of the study, the procedures involved, the potential risks and benefits, and their right to withdraw at any time without affecting their medical care.

Consent for Publication

All authors have reviewed and approved the final version of the manuscript and have given their consent for publication. The work is submitted for publication under the Creative Commons Attribution Non-Commercial 4.0 license, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Data Availability

The datasets generated and analysed during the current study, including de-identified participant data, genotype information, and serum MBL measurements, are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy and ethical restrictions, but can be obtained from the corresponding author, subject to institutional data sharing agreements and approval from the relevant ethics committees.

Competing Interests

The authors declare that they have no competing interests, financial or non-financial, that could have influenced the work reported in this paper. No author has any financial or personal relationships with other people or organisations that could inappropriately influence or bias the content of the paper.

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Author Contributions

ADG conceived and designed the study, supervised the entire research project, and secured the necessary resources and funding for its execution.

MBJ performed the formal statistical analysis, curated the data, and created the visualisations.

ADG and MBJ contributed to the development of the methodology, carried out the laboratory investigations, participated in the validation of results, collaborated on drafting the original manuscript, and subsequently reviewed and edited it for important intellectual content.

All authors have read and approved the final version of the manuscript for publication. All authors have read and agreed to the published version of the manuscript.

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