Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June/ 2026
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Association of STAT3 rs744166 and JAK2 rs10758669 Polymorphisms with Coeliac Disease Susceptibility in an Iraqi Population

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Received: 5/4/2026, Accepted: 12/5/2026, Published: 30/6/2026.

Abstract

Coeliac disease (CD) is one of the autoimmune illnesses caused by ingestion of diets including gluten. The immune cell activation and tolerance regulated via Janus kinase2 and the single transducer and activator of transcription3 (JAK-STAT signaling pathway) While HLA-DQ8 and HLA-DQ2 are well established genetic risk factors for coeliac disease, non-HLA polymorphisms may contribute to disease susceptibility in different populations. This study examined the relationship between genetic variation within STAT3 (rs744166) and JAK2 (rs10758669) with the susceptibility of coeliac disease. forty sample were collected from Peripheral blood 20 samples were collected from patients with biopsy-confirmed coeliac disease and 20 blood samples from healthy controls (age and sex matched). The extraction of Genomic DNA and performance of SNP genotyping by using a real-time PCR kit provided by Thermo-Fisher Scientific. Allelic and genotypic frequencies were compared between patients and controls to assess potential associations. The first human SNP of JAK2 rs10758669 is part of the JAK–STAT signaling pathway, which represented three genotypes (CC, AC, and CC), The CC genotype indicated significant differences (P-Value 0.006), while other genotypes showed no significant differences. The second human SNP of STAT3 rs744166 also showed three genotypes (AA, GG, and AG). The AG genotype represented a significant difference (p- value 0.025), while other genotypes (AA, AG) showed no significant differences. The study finds that STAT3 (rs744166) and JAK2 (rs10758669) are significantly associated with coeliac disease and these SNPs could be used as a biomarker.

Keywords: Coeliac disease, autoimmune disorders, JAK-STAT signaling pathway, JAK2 (rs10758669), STAT3 (rs744166).

Introduction

Coeliac disease is a one autoimmune disorders induced intestinal inflammation after intake of gluten containing diet in people with genetic susceptibility, CD approximately affecting 1% of the population worldwide in individuals (globally, Coeliac disease affecting about 3 million humans) [1,2,3]. People with Coeliac disease have high sensitivity to gluten protein that may be found in barely, wheat,

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and rye after ingestion of gluten. The cells of intestine are injury and damaged as a result for the overactivation of the immune system, which caused several symptoms that may be present in CD patients like fatigue, diarrhea, weight loss, and other undesirable symptoms [3,4].

Individual with CD may exhibit gastrointestinal and extraintestinal symptoms, or they mayn't exhibit any symptoms at all. Approximately 50% of Coeliac patients showed extraintestinal symptoms like Anemia, Dental Enamel Hypoplasia, Neurological problems, and Osteoporosis. CD is multifactorial disorder due to have genetical and immunological bases. Also, the type of diet has big effect on stimulation of the CD because the ingestion of gluten containing diets caused mucosal injury of small intestine leading to variety of clinical aspects [5,6].

The diagnosis of coeliac disease involves many serological tests for antibodies like tissue transglutaminase (tTG) [7,8]. A gluten free diet is essential for the reliable measurement for each of these antibodies. As a result, test results are used to gauge therapy effectiveness and dietary compliance [9,10]. Untreated symptomatic disease is associated with higher morbidity and death, accompanying a worsening in quality of life. Poor growth, failure to thrive, vitamin shortages, persistent weariness, and gastrointestinal distress are just a few of the many clinical manifestations [11,12,13].Numerous physiological processes, including cell stress, growth, proliferation, differentiation, and apoptosis, are mediated by the Janus kinase2- signal transducer and activator of transcription3 signaling pathway, which is involved in the signal transduction pathway of cells [7,8,11]. The purpose of this article is to investigate the association between JAK2/STAT3 pathway with coeliac disorder in Iraqi population.

Methodology

Subjects:

The current case control study including 40 sample diagnosed by physician (20 patients and 20 healthy controls), samples were collected from private hospital in Baghdad/Iraq (samples collection extends about 4 months from January to May 2025). The study includes people who are diagnosed with coeliac disease and have positive serological marker for anti-tissue transglutaminase antibody, while excluded the patients who have other gastrointestinal disorders, pregnant and lactating women and the patient that couldn't complete the study procedure or follow-up.

DNA Extraction:

For the extraction of DNA 5 ml of patients peripheral blood was collected from in EDTA tube. And then DNA isolated by using commercial kit for DNA extraction provided by zymo research (D3025, Quick-DNA Miniprep, USA)[14]. Furthermore, Concentration and purity of DNA were evaluated by using NanoDrop spectrophotometry, Samples with A260/A280 ratios between 1.7 and 2.0 were included in the analysis.

Genotyping of rs744166 and rs10758669 Using TaqMan Assays

Genotyping of rs744166 and rs10758669 polymorphisms was accomplished by using TaqMan allelic discrimination tests (Thermo Fisher Scientific company\ USA). The validated SNP-specific TaqMan assays C___3140282_10 (for rs744166) and C___2008279_10 (for rs10758669) were selected based on their compatibility with the human genome and their proven analytical performance in previous studies[15].

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A total volume of 20 µL was used to prepare each real _time PCR reaction, which included 10 µL of TaqMan Genotyping Master Mix, 0.5 µL of SNP genotyping assay, a specific size of each eluted DNA sample equal to concentration 10_20 ng, and nuclease-free water to finish the reaction volume up to 20 µL. The Sacace Sacylcer-96 real-time PCR machine was used to perform amplification and allelic determination using the subsequent thermal cycling program: forty cycles of denaturing at 95°C for fifteen second intervals and annealing/extension at 60°C for one minute, then follow the first enzyme stimulation step at 95°C for 10 minutes [16].

Statistical analysis

The independent groups Student's T-test was used to compare continuous factors, which were reported as average (SD). When any predicted cell count was less than five, Fisher's exact test or Pearson's chi-square χ^2 test were used for comparing the categorical factors, which were reported as frequency values and percent. To determine odds ratios (ORs) and confidence intervals (CIs) of 95% under genotypic, inherited, recessive, and allelic models, allele frequencies and genotypes between coeliac patients and healthy controls were examined. Additionally, Hardy-Weinberg equilibrium (HWE) 15 was used to evaluate each SNP in the control group. all tests were two-tailed, and statistical significance was defined as $p < 0.05$[17,18].

Results and Discussions

According to the data analysis of forty blood samples gives current results where the mean age was 30.25 ± 7.95 years for patients and 30.70 ± 6.21 years for control groups as shown in Table (1). 60% and 55% (for the patients and control groups, respectively) is the percentage of females who contributed to the current study, whereas the male percentage was 40 % and 45 % for the patients and the control groups. These results present no significant difference between CD patient and control groups where the P-value is (0.83) and (0.75) for age and gender, respectively. 45% of patients have Family history of coeliac disease and 55% without family history while controls group have 100% without family history and this result shows significant differences between CD patient and control groups where the P-value is (< 0.001). 100% of patients have symptoms and give positive results for Anti-tTG while healthy control group don't have any symptoms and give negative results for Anti-tTG there for the results shows high significant differences P-value (< 0.0001) between CD patient and control groups.

Table 1. Demographic, clinical and serological characteristics of coeliac patients and controls

Variable	**Patients (n = 20)**	**Controls (n = 20)**	**Statistical Test**	**p-value**
Age (years), mean ± SD	30.25 ± 7.95	30.70 ± 6.21	Student's t-test	**0.83**
Sex	Female: 12 (60%)	Female: 11 (55%)	Chi-square	**0.75**
	Male: 8 (40%)	Male: 9 (45%)		
Family history of coeliac disease	Yes: 9 (45%)	Yes: 0 (0%)	Fisher's Exact	**< 0.001**
	No: 11 (55%)	No: 20 (100%)		
Main clinical presentation	Symptomatic: 20 (100%)	Symptomatic: 0	Fisher's Exact	**< 0.0001**

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	Asymptomatic: 0	Asymptomatic: 20 (100%)		
Anti-tTG status	Positive: 20 (100%)	Positive: 0	Fisher's Exact	< **0.0001**
	Negative: 0	Negative: 20 (100%)		

For the majority of participants in this investigation, family history is a major factor in the emergence of coeliac disease[١٩], and the findings are corroborated by research of Can et al.[٢٠]. The first step in diagnosing coeliac disease in suspected individuals is a serological study. The most useful serological examination is the IgA anti-tissue transglutaminase reagent (anti TTG-IgA), which has more specificity and sensitivity than other tests using antibodies [٢١,22,23]. Patients with a positive anti-TTG-IgA should have an intestine biopsy for a definite diagnosis of coeliac disease, as the results of the present study supported other worldwide research [24,25] where the result showed high significant difference in anti- tTG between CD patients and control groups.

For rs744166, a significant deviation from HWE was observed (p = 0.007). The observed genotype distribution showed a deficiency of the homozygous GG genotype compared to the expected values, accompanied by an excess of heterozygous AG individuals. In contrast, the rs10758669 genotype distribution was consistent with HWE (p = 0.439), indicating no statistically significant difference between observed and expected genotype frequencies (Table 2).

Table 2. Hardy Weinberg equilibrium of SNP rs744166 and rs10758669

SNP	**Genotype**	**Observed (O)**	**Expected (E)**	**chi-square**	**P-Value (df = 1)**
rs744166	**AA**	5	7.812	**7.197**	**0.007**
	AG	15	9.375		
	GG	0	2.812		
rs10758669	**AA**	7	7.812	**0.599**	**0.439**
	AC	11	9.375		
	CC	2	2.812		

The human JAK2 rs10758669 polymorphism, which has three genotypes (CC, AC, and AA) that match two alleles (A and C), is a crucial part of the JAK–STAT signaling system. Three genotypes—CC, AC, and AA—represent 15%, 35%, and 50% of CD patients' genotypes in the current study, in comparison to 35%, 55%, and 10% of healthy controls. Other genotypes did not exhibit any significant changes, while the CC genotype did (P-Value 0.006). The two alleles' frequencies (the A and C alleles) showed significant variations with a p-value of 0.013 (Table 3).

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Table 3. Genotype and allele distribution of rs10758669 and their association with coeliac disease

rs10758669					
Group	Patients (n=20)	Controls (n=20)	Chi-square	OR	P-Value
AA n (%)	3 (15%)	7 (35%)	2.13	0.33	0.14
AC n (%)	7 (35%)	11 (55%)	1.62	0.44	0.2
CC n (%)	10 (50%)	2 (10%)	7.62	9	0.006
A allele (n, %)	13 (32.5%)	25 (62.5%)		3.46	0.013
C allele (n, %)	27 (67.5%)	15 (37.5%)			

The JAK–STAT signaling pathway depends on the human SNP of STAT3 rs744166, which represents 3 genotypes (AA, AG, and GG) that relate to 2 alleles (A and G). 3 genotypes—AA, AG, and GG—accounted for 50%, 40%, and 10% of CD patients' genotypes in the current investigation, as opposed to 25%, 75%, and 0% of healthy controls. Other genotypes did not exhibit significant variations, however the AG genotype did (p-value 0.025). There were no significant differences between the allele frequencies of the two alleles (A and G alleles), with a p-value of 0.637 (Table 4).

Table 4. Genotype and allele distribution of rs744166 and their association with coeliac disease

rs744166					
Group	**Patients (n=20)**	**Controls (n=20)**	**Chi-square**	**OR**	**P-Value**
AA n (%)	10 (50%)	5 (25%)	**2.67**	**3**	**0.102**
AG n (%)	8 (40%)	15 (75%)	**5.01**	**0.22**	**0.025**
GG n (%)	2 (10%)	0 (0%)	0	∞	**0.487**
A allele (n, %)	28 (70%)	25 (62.5%)		**0.71**	**0.637**
G allele (n, %)	12 (30%)	15 (37.5%)			

The association between rs10758669 genotypes and clinical response to a gluten-free diet in coeliac patients Among patients showing an improved response (n = 16), the CC genotype was the most frequent 50%, followed by AC 37.1% and AA 12.5%. In contrast, among patients with a partial response (n = 2), genotypes AA and CC were equally represented 50%, while the AC genotype was not observed in this group and the result showed no significant differences between rs10758669 genotypes and clinical response to a gluten-free diet where p-value 0.314.

The analysis of STAT3 rs744166 and JAK2 rs10758669 polymorphisms presented a significantly higher frequency of the AG genotype for rs744166 and CC genotype for rs10758669 in the CD patients group compared to controls. These findings were compatible with the results reported by Medrano et al. [1], who revealed that JAK2 rs744166 and STAT3 rs744166, two SNPs recently linked to Crohn's disease, share some vulnerability characteristics with coeliac disease [24,26]. Additionally, when matching the current findings to those of other research that investigated different autoimmune illnesses, they were consistent with those findings [19,25].

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Also, the result showed no significant differences between rs744166 genotypes and clinical response to a gluten-free diet where p-value 0.471 where patients showed an improved response (n = 16), the AA genotype was the most frequent 56.3%, followed by AG 37.5% and GG 6.3%. In contrast, among patients with a partial response (n = 2), genotypes AG represented 100%, while the AA and GG genotypes were not observed in this group. The JAK2 rs744166 and STAT3 rs744166 polymorphisms and the medical reaction to a gluten-free diet in coeliac patients were examined in this study. Although variations in genotype distribution were observed between patients with improved and partial responses, the association did not reach statistical significance, suggesting that JAK2 rs744166 and STAT3 rs744166 alone may not be a strong determinant of dietary response in this cohort that could be due to the environment and lifestyle factors.

Table 5. Association between rs10758669 and rs744166 genotypes and clinical response to a gluten-free diet in coeliac patients

rs10758669			**rs744166**		
Response	Improved	Partial	**Response**	Improved	Partial
AA	2 (12.5%)	1 (50.0%)	**AA**	9 (56.3%)	0 (0.0%)
AC	6 (37.5%)	0 (0.0%)	**AG**	6 (37.5%)	2 (100.0%)
CC	8 (50.0%)	1 (50.0%)	**GG**	1 (6.3%)	0 (0.0%)
Total	16 (100%)	2 (100%)	**Total**	16 (100%)	2 (100%)
P-Value	0.314		P-Value	0.471	

Conclusion

This study presents a significant association between JAK2- STAT3 signaling pathway (STAT3 (rs744166) and JAK2 (rs10758669)) and susceptibility to coeliac disease in the Iraqi patients. The study suggests a potential role of these SNPS as genetic factors where the results of present study showed a significant difference among patients and control groups for the CC genotype of JAK2 rs10758669 and the AG genotype of STAT3 rs744166. Also, the study presents no significant association between these polymorphisms and how patients react to a gluten-free diet, albeit more research with a bigger sample size is advised to validate these findings.

Acknowledgments

"The author is grateful to the Department of Medical and Molecular Biotechnology, AL-Nahrain University for their support."

Author's Declaration

- We so attest that every table in the document is unique and was made by us.
- The local ethical committee at [Al-Nahrain University, College of Biotechnology] has granted us ethical clearance for our work. This approval demonstrates our dedication to participating welfare and ethical research methods.

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Published Online First: 30/ June/ 2026
p-ISSN: 3007-5491 / e-ISSN:3007-5505

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