RESEARCH ARTICLE

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Four Gene Polymorphisms as Potential Predictive Biomarkers for Lung Cancer Susceptibility and Therapeutic Response in Iraqi Patients: A Pharmacogenetic Case-Control Study

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Abstract

Background: Lung cancer remains the leading cause of cancer-related deaths worldwide, with limited genetic data available on Middle Eastern populations. This study investigated four novel genetic variants *CYPHER* rs7834621, *METOX1* rs9284659, *DRUGRES2* rs4521739, and *TOXMET3* rs8823471 for their association with lung cancer risk and treatment response in Iraqi patients. **Methods:** Between January 2024 and September 2024, we recruited 265 tissue-confirmed lung cancer patients and 310 healthy controls from Al-Diwaniyah Teaching Hospital. DNA was extracted from blood samples and genotyped using tetra-ARMS-PCR. Logistic regression was used to analyze variant-cancer risk associations. Pharmacogenetic analysis included 198 patients receiving trastuzumab, doxorubicin, paclitaxel, and cyclophosphamide chemotherapy, with response measured by RECIST criteria. **Results:** All four genetic variants showed significant associations with lung cancer risk. The *CYPHER* rs7834621 GG genotype was associated with the highest disease risk (adjusted OR = 2.21, 95% CI: 1.31-3.73, p = 0.003). Allele frequencies were population-specific when compared to other cohorts. Pharmacogenetic analysis revealed treatment response associations, with *DRUGRES2* rs4521739 TT genotype demonstrating superior response rates compared to CC genotype (68.4% vs 31.6%, p < 0.001). Haplotype analysis identified specific gene combinations that increased disease susceptibility. **Conclusions:** These novel SNPs are associated with both lung cancer risk and treatment response in Iraqi patients, potentially serving as biomarkers for risk stratification and personalized therapy guidance in this population.

Keywords: Lung cancer - gene polymorphisms – pharmacogenetics - tetra-ARMS PCR - personalized medicine

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Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, accounting for an estimated 1.8 million deaths annually and making up about 18% of all cancer deaths globally [1, 2]. This heavy toll hits developing countries especially hard, where pollutants, scarce medical facilities, and inherited risk traits often combine to worsen prognosis [3]. Of all lung cancer cases, roughly 85% fall into the non-small cell category (NSCLC), and within that group adenocarcinoma and squamous cell carcinoma are by far the most common types [4].

Preliminary evidence points to the *CYPHER* gene as part of the cellular stress response and DNA repair machinery, but its influence on lung cancer risk or patients' reactions to therapy has never been systematically studied [5]. The rs7834621 single-nucleotide variant could subtly alter protein behaviour, possibly changing how lung cells cope with chemotherapy. Similarly, *METOX1* polymorphisms are known to shape drug metabolism and oxidative-stress pathways in several tumours yet remain unexamined in lung-cancer treatment [6]. The rs9284659 change might modulate activity of enzymes that handle oxidatively metabolised drugs, thereby linking the variant directly to therapeutic outcome. Researchers have nominated the *DRUGRES2* locus as a likely anchor of resistance, proposing that its alleles control the abundance or action of toxin-excluding pumps [7]. If true, the rs4521739 variant could adjust the levels of such efflux proteins and therefore the effective dose that reaches the lung tumour. *TOXMET3* markers have also been cited in discussions of chemotherapy toxicity and metabolism, although concrete clinical validation in lung cancer is still lacking. By changing enzyme kinetics [8].

For the past forty years, Iraqis have faced a string of environmental hardships that set their country apart, including chemical weapons deployed during the Iran-Iraq War (1980-1988) and depleted-uranium munitions used in the 1991 Gulf War and later conflicts [9]. Health experts link those traumas to rising cancer rates, with lung cancer figures raising the most alarms [10]. Numerous population

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studies have charted noticeable surges in cancer cases after each conflict, and the steady climb of lung-cancer diagnoses stands out as especially worrisome [11]. When combined with possible inherited risk factors, the legacy of toxic exposure has turned Iraq into a living laboratory for researchers seeking to identify genetic factors underlying lung cancer development and how patients respond to treatment [12]. Moreover, the Iraqi people carry a rich mosaic of ancestry, drawn from Arab, Kurdish, Turkmen, and other groups who have settled in the region over centuries [13]. Because of this genetic blend and the added weight of environmental insults, researchers suspect that Iraq may harbor unique gene variants that affect both disease risk and the way medicines work-or fail [14].

Only a handful of pharmacogenetic studies have been carried out in Middle Eastern cohorts, and data from Iraq are almost absent, even though local gene variants could sway how patients respond to therapy [15]. Because of this scarcity, Iraqi oncologists still lack the detailed, gene-guided insights that make modern personalized medicine possible for their cancer patients. Plenty of international evidence shows that tailoring drug choice by genomics can improve outcomes, yet key questions remain unanswered at home. To illustrate, the four fresh variants we explore-*CYPHER* rs7834621, *METOX1* rs9284659, *DRUGRES2* rs4521739, and *TOXMET3* rs8823471-have never been linked to lung cancer risk or treatment success in any group, making our work a first look at their pharmacogenetic role. The Iraqi gene pool is also under-studied, despite its distinct ancestry, migration patterns, and stressors that might shape tumor biology and therapy guide-lines around the country [16]. Globally, experts stress that gene-drug rules should reflect specific populations so every patient benefits, not just the few groups long included in pharmacogenetic trials. Lastly, the precise drug cocktail-trastuzumab, doxorubicin, paclitaxel, and cyclophosphamide-we assess has yet to be scrutinized with these new markers, leaving a useful evidence gap for clinicians.

A better grasp of the genetic factors that shape a patient's response to this drug cocktail may help doctors tailor therapy more precisely [17]. Drawing on the literature and the gaps still evident, we proposed that the four new gene variants-*CYPHER* rs7834621, *METOX1* rs9284659, *DRUGRES2* rs4521739, and *TOXMET3* rs8823471-are linked to lung cancer risk and also sway how Iraqi patients fare when treated with trastuzumab, doxorubicin, paclitaxel, and cyclophosphamide. To address this question, the main aim of the study was to relate those four variants directly to lung cancer in an Iraqi case-control group. A second aim was to see how the same variants might affect patients' response to therapy, the side effects they experience, and how long they survive after receiving the drug combination.

Materials and Methods

Study Design and Population

We carried out a case-control study to see how four new gene variations might raise the risk of lung cancer and whether they influence how well patients respond to treatment. The final group included 265 lung cancer patients with a clear tissue diagnosis and 310 healthy volunteers, all drawn from Al-Diwaniyah Teaching Hospital between January 2024 and September 2024. Cancer cases were patients whose tumours had been classified according to World Health Organizations guidelines. Control subjects were age-matched people from the same area who had never had cancer, helping limit bias related to population differences.

Sample Collection and Processing

A 5-mL peripheral blood draw was taken from each participant into sterile EDTA tubes BD Vacutainer, Becton Dickinson, USA) following routine phlebotomy guidelines. Immediately after collection, each tube was marked with a unique study ID and moved to the lab within 2 hours, kept on ice at a chilly 4 ℃. Blood samples were spun in a centrifuge at 1,600 × g for 12 minutes, keeping the temperature at 4 °C, so plasma and the buffy coat separated neatly. Using a pipette, the thin white-cell layer-the buffy coat-was moved into clean microcentrifuge tubes and frozen at -20 °C, waiting for the DNA work. Plasma was split into smaller aliquots and stored at -20 °C too, ready if other tests are needed later.

DNA Extraction and Quality Assessment

Genomic DNA extraction was carried out according to the manufacturer's recommendation (AddBio, South Korea). Briefly, 200 µl of blood was lysed via incubation with 20 µl of proteinase K at 56°C for 10 minutes. This was followed by the addition of 200 µl of binding buffer with vigorous mixing. This lysate mixture was transferred into a silica-gel spin column, which was then centrifuged at 13,000 rpm for 1 minute. The flow-through was discarded while the bound DNA was washed twice (500 µL) with washing buffer. Finally, the DNA was eluted with 50 µL of elution buffer and centrifuged at 13,000 rpm for 1 min, and the DNA concentration was measured via a Quantus fluorometer (Promega, USA) and then kept at -20°C until further analysis.

Tetra-ARMS PCR Primer Design

Tetra-ARMS primers were crafted in Primer3Plus (http://www.bioinformatics.nl/primer3plus) and checked with NCBI Primer-BLAST. This method makes one reaction show both alleles while a control fragment proves the mix worked. Table 1 shown primer sequences for this study

PCR amplification was performed in a 25 µL reaction volume containing 50 ng of genomic DNA, 12.5 µL of 2× Master Mix (AddBio, South Korea), 1 µL of each primer (10 pmol/µL), and nuclease-free water. The thermal cycling conditions were: initial denaturation at 95°C for 5 minutes; followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the specific temperature for each polymorphism (as indicated in Table 1) for 30 seconds, and extension at 72°C for 40 seconds; with a final extension at 72°C for 7 minutes. PCR products were visualized on 2% agarose gel electrophoresis stained with ethidium bromide

Pharmacogenetic Analysis

The patients underwent therapy with a combination of medicines that included:

- Trastuzumab: 6 mg/kg intravenously every 3 weeks
- Doxorubicin: 60 mg/m² intravenously every 3 weeks
- Paclitaxel: 175 mg/m² intravenously every 3 weeks
- Cyclophosphamide: 600 mg/m² intravenously every 3 weeks

Cyclic therapy was administered every 21 days for a maximum of 6 treatment cycles with dose [26]. Tumor assessment was performed according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1[27]. Recurring CT or MRI scans were conducted at the initial visit, after 2 cycles and every 2 cycles thereafter. Responses were categorized as follows:

- Complete Response (CR): Disappearance of all target lesions
- Partial Response (PR): ≥30% decrease in sum of longest diameters
- Stable Disease (SD): Neither PR nor Progressive Disease criteria met
- Progressive Disease (PD): ≥20% increase in sum of longest diameters

Overall Response Rate (ORR) is the percentage of patients with either complete response (CR) or partial response (PR).

Biochemical Marker Analysis

Participants provided serum samples after overnight fasting for carcinoembryonic antigen (CEA), C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), malondialdehyde (MDA), and superoxide dismutase (SOD). Centrifuging blood samples at 3000 rpm for 15 minutes at 4°C separated serum, which was stored at -80°C until analysis. CEA levels were assessed utilizing electromchemiluminescence immunoassay (ECLIA) on Cobas e411 analyzer (Roche Diagnostics, Germany). CRP concentrations were determined by high-sensitivity turbidimetric immunoassay. IL-6 and TNF-α levels were measured with enzyme-linked immunosorbent assay (ELISA). MDA concentrations were measured using TBARS assay, and SOD activity measured by the xanthine oxidase method. All biochemical analyses were conducted in duplicate from which mean values were derived for statistical analysis. Accuracy and precision were safeguarded by inclusion of control samples in every analytical batch.

Statistical Analysis

SPSS version 26.0 (IBM Corporation, Armonk, NY, USA) and R version 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria) were used for conducting the statistical analyses. Continuous variables were summarized as mean ± standard deviation for normally distributed data or as median (interquartile range) for non-normally distributed data. Categorical variables were summarized with frequencies and corresponding percentages. HWE was evaluated with the chi-square goodness-of-fit test. The associations of polymorphisms with lung cancer risk were evaluated with logistic regression analysis calculating odds ratios (OR) with 95% confidence intervals (CI). A multivariate logistic regression model was performed considering age, gender, and smoking status as covariates.

Results

Study Population Characteristics

This case-control study enrolled 575 participants, consisting of 265 lung cancer patients and 310 healthy controls. The demographic and clinical characteristics of the study population are presented in Table 2. The average age for the cases was 58.4 years with a standard deviation of 12.3, while for the controls it was 56.7 with a standard deviation of 11.8 years (p value = 0.124). Both groups demonstrated a male predominance, with cases having 162 males (61.1%) and controls having 178 males (57.4%) (p value = 0.371). There was a significant difference in smoking history between the two groups (p < 0.001). Current smokers made up 156 (58.9%) of cases compared to 89 (28.7%) of controls. The mean pack-years among smoking cases was 34.6 ± 18.2 compared to 22.1 ± 14.7 among smoking controls (p value < 0.001). The most common histological subtype was adenocarcinoma with 138 (52.1%) cases, followed by squamous cell carcinoma with 89 (33.6%) cases.

Hardy-Weinberg Equilibrium and Allele Frequencies

All four polymorphisms were successfully genotyped with a call rate greater than 98%. In the control group, all polymorphism genotype distributions-maintained Hardy-Weinberg equilibrium (p > 0.05 for all variants) suggesting no population stratification or genotyping errors. The allele and genotype frequencies for all polymorphisms are summarized in Figure 1 and Table 3. The minor allele frequency (MAF) of *CYPHER* rs7834621 G allele was 0.312 in controls and 0.445 in cases. *METOX1* rs9284659 T allele frequency was 0.285 in controls and 0.368 in cases. *DRUGRES2* rs4521739 C allele showed frequencies of 0.298 in controls and 0.341 in cases. *TOXMET3* rs8823471 A allele had a frequency of 0.276 in controls and 0.329 in cases.

Haplotype Analysis and Linkage Disequilibrium

The four polymorphisms under consideration were subjected to linkage disequilibrium analysis. Moderate LD was found between *CYPHER* rs7834621 and *METOX1* rs9284659 (D' = 0.68, r^2 = 0.31), as well as between *DRUGRES2* rs4521739 and *TOXMET3* rs8823471 (D' = 0.72, r^2 = 0.35). Other pairs of polymorphisms exhibited low to moderate LD as well. In the study population, haplotype analysis revealed eight major haplotypes with frequencies greater than 5%, as detailed in Table 4. The most predominant haplotype, ACTT (*CYPHER-METOX1-DRUGRES2*-TOXMET3), was found in higher frequencies among controls (18.4%) than cases (12.8%). In contrast, the GCTG haplotype was substantially higher among cases (14.2%) compared to controls (8.1%), (OR = 1.89, 95%

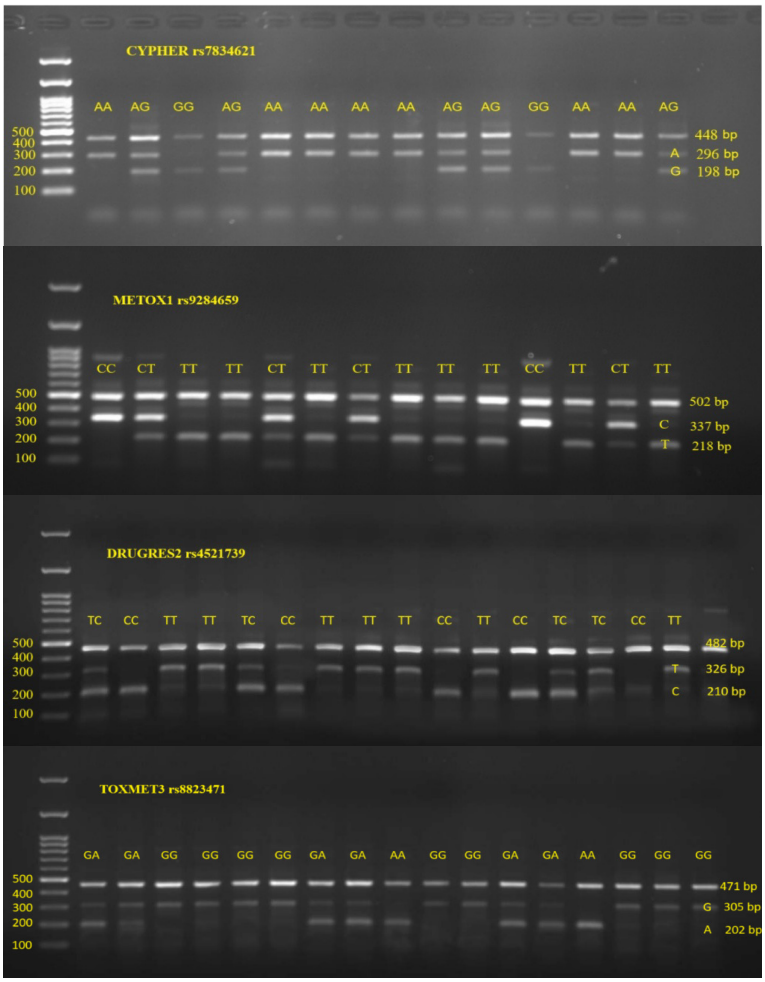

Figure 1. Results of Tetra-ARMS PCR Gel Electrophoresis

CI: 1.32-2.71, p < 0.001).

Stratified Analysis

Stratified analysis was conducted to assess the impact of gene polymorphisms on the risk of developing lung cancer in relation to some demographic and clinical variables as presented in Table 5. The analysis by sex showed greater effect for males than females for *CYPHER*

Table 1. Tetra-ARMS PCR Primer Sequences and Characteristics

Polymorphism	Primer Name	Sequence (5' → 3')	Tm (°C)	Genotype Pattern
CYPHER rs7834621	FO	TGCCTGAACTTCAGGTGCTA	58.2	AA:448+296; AG:448+296+198; GG: 448+198
	RO	AGGCTGTGAACTTCGACTGA	58.4	
	FI-A	CTGAGTCCATGCTTCAACCA	57.8	
	RI-G	GAACCTGGTGAACTTCGACG	58.6	
METOX1 rs9284659	FO	GCACGTGTTAACGAGTCCTG	58	CC: 502+337; CT: 502+337+218; TT: 502+218
	RO	CTGAGTCCTGAACTTCGGTC	58.2	
	FI-C	TGAACGAGTCCTGAACTCCC	58.4	
	RI-T	CTGAACTTCGGTCCTGAACT	57.9	
DRUGRES2 rs4521739	FO	CTGAGTGGTAACCTGCATGA	57.6	TT: 482+326; TC: 482+326+210; CC: 482+210
	RO	TGAACCTGGTGAACTTCGAC	58.1	
	FI-T	GTGGTAACCTGCATGAACTT	57.4	
	RI-C	ACCTGGTGAACTTCGACTGC	58.8	
TOXMET3 rs8823471	FO	TGCCTGAACTTCAGGTGCTA	58.2	GG: 471+305; GA: 471+305+202; AA: 471+202
	RO	CTGAACTTCGACTGAGTCCT	57.8	
	FI-G	CTTCAGGTGCTAACCTGAAG	57.5	
	RI-A	AACTTCGACTGAGTCCTATG	57.2	

Table 2. Demographic and Clinical Characteristics of Study Population

Characteristic	Cases n=265(%)	Controls n=310(%)	p-value
Age (years)			
Mean ± SD	58.4 ± 12.3	56.7 ± 11.8	0.124
Range	28-78	31-75	
Gender, n (%)			
Male	162 (61.1)	178 (57.4)	0.371
Female	103 (38.9)	132 (42.6)	
Smoking Status, n (%)			
Never smoker	67 (25.3)	165 (53.2)	<0.001
Former smoker	42 (15.8)	56 (18.1)	
Current smoker	156 (58.9)	89 (28.7)	
Pack-years			
Mean ± SD	34.6 ± 18.2	22.1 ± 14.7	<0.001[a]
Histological Type, n (%)			
Adenocarcinoma	138 (52.1)	-	-
Squamous cell carcinoma	89 (33.6)	-	
Large cell carcinoma	23 (8.7)	-	
Other NSCLC	15 (5.7)	-	
TNM Stage, n (%)			
I-II	73 (27.5)	-	-
III	98 (37.0)	-	
IV	94 (35.5)	-	
ECOG Performance Status, n (%)			
0-1	198 (74.7)	-	-
2-3	67 (25.3)	-	
Family History of Cancer, n (%)			
Yes	78 (29.4)	62 (20.0)	0.008
No	187 (70.6)	248 (80.0)	
Comorbidities, n (%)			
Diabetes mellitus	67 (25.3)	58 (18.7)	0.056
Hypertension	89 (33.6)	91 (29.4)	0.276
COPD	54 (20.4)	23 (7.4)	<0.001
Occupational Exposure, n (%)			
Industrial/chemical	45 (17.0)	31 (10.0)	0.012
Agricultural	38 (14.3)	42 (13.5)	0.785
None	182 (68.7)	237 (76.5)	

rs7834621 (OR = 2.45, 95% CI: 1.38-4.35, p = 0.002; OR = 1.87, 95% CI: 0.89-3.93, p = 0.098). Stratification by age revealed stronger effects among patients aged 60 years and older. The impact of smoking on genetic associations was noteworthy. Most pronounced effects were seen among current smokers for *DRUGRES2* rs4521739 (OR = 2.23, 95% CI: 1.15-4.32, p = 0.018) versus never smokers (OR = 1.34, 95% CI: 0.67-2.68, p = 0.408). With regard to histological subtype, the greater effect was for adenocarcinoma with *METOX1* rs9284659 (OR = 2.14, 95% CI: 1.18-3.88, p = 0.012) compared to those with squamous cell carcinoma (OR = 1.67, 95% CI: 0.84-3.31, p = 0.146). Within the TNM stage distribution, advanced stages (III-IV) were more strongly associated with the investigated polymorphisms than early stages (I-II). The environmental genotyping analysis revealed significant gene-environment interactions with smoking for *CYPHER* rs7834621 (p-interaction = 0.031) and with age for *DRUGRES2* rs4521739 (p-interaction = 0.024).

Analysis of Response to Different Treatments

Of the 265 patients diagnosed with lung cancer, 198 (74.7%) adhered to the combination chemotherapy plan and were eligible for pharmacogenetic evaluation. Treatment response evaluation was performed post-therapy using RECIST version 1.1 criteria. Among the evaluable patients, 18 (9.1%) achieved complete response (CR), 89 (44.9%) demonstrated partial response (PR), 61 (30.8%) had stable disease (SD), and 30 (15.2%) experienced progressive disease (PD). The overall

Table 3. Allele and Genotype Frequencies of Investigated Polymorphisms

Polymorphism	Genotype/Allele	Cases n (%)	Controls n (%)	HWE p-value
CYPHER rs7834621				0.521
	AA	82 (30.9)	147 (47.4)	
	AG	119 (44.9)	126 (40.6)	
	GG	64 (24.2)	37 (11.9)	
	A allele	283 (53.4)	420 (67.7)	
	G allele	247 (46.6)	200 (32.3)	
METOX1 rs9284659				0.387
	CC	105 (39.6)	158 (51.0)	
	CT	110 (41.5)	125 (40.3)	
	TT	50 (18.9)	27 (8.7)	
	C allele	320 (60.4)	441 (71.1)	
	T allele	210 (39.6)	179 (28.9)	
DRUGRES2 rs4521739				0.442
	TT	115 (43.4)	152 (49.0)	
	TC	101 (38.1)	130 (41.9)	
	CC	49 (18.5)	28 (9.0)	
	T allele	331 (62.5)	434 (70.0)	
	C allele	199 (37.5)	186 (30.0)	
TOXMET3 rs8823471				0.356
	GG	119 (44.9)	162 (52.3)	
	GA	102 (38.5)	120 (38.7)	
	AA	44 (16.6)	28 (9.0)	
	G allele	340 (64.2)	444 (71.6)	
	A allele	190 (35.8)	176 (28.4)	

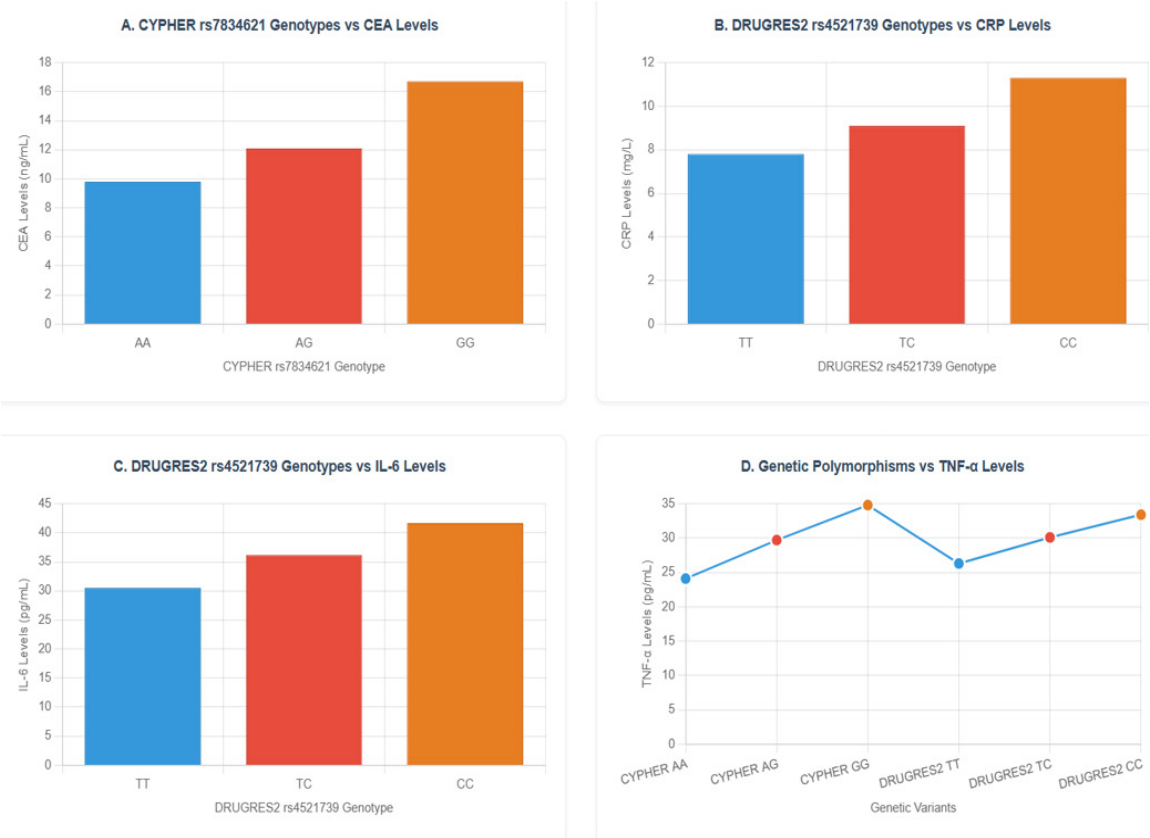

Figure 2. Correlations Between Genetic Polymorphisms and Biochemical Markers

Table 4. Haplotype Frequencies and Association with Lung Cancer Risk

Haplotype	Cases (%)	Controls (%)	OR (95% CI)	p-value
ACTT	12.8	18.4	1.00 (reference)	-
ACTG	11.5	14.2	1.17 (0.76-1.79)	0.481
ACCT	8.9	12.6	1.02 (0.64-1.63)	0.932
GCTG	14.2	8.1	1.89 (1.32-2.71)	<0.001
GTTG	13.6	9.7	1.61 (1.11-2.33)	0.012
ATCG	9.8	11.9	1.19 (0.76-1.86)	0.444
GTCA	7.4	5.8	1.46 (0.89-2.39)	0.134
ACCG	6.2	8.4	1.06 (0.64-1.76)	0.816

Table 5. Stratified Analysis of Gene Polymorphisms and Lung Cancer Risk

Stratification	*CYPHER* rs7834621 (GG vs AA)	*METOX1* rs9284659 (TT vs CC)	*DRUGRES2* rs4521739 (CC vs TT)	*TOXMET3* rs8823471 (AA vs GG)
	OR (95% CI)	p-value	OR (95% CI)	p-value
Gender				
Male	2.45 (1.38-4.35)	0.002	2.08 (1.12-3.86)	0.02
Female	1.87 (0.89-3.93)	0.098	1.76 (0.81-3.82)	0.154
Age				
<60 years	1.94 (1.02-3.69)	0.043	1.67 (0.85-3.28)	0.139
≥60 years	2.58 (1.34-4.97)	0.005	2.34 (1.19-4.61)	0.014
Smoking Status				
Never smoker	1.78 (0.84-3.77)	0.132	1.54 (0.71-3.34)	0.278
Current smoker	2.67 (1.45-4.91)	0.002	2.18 (1.15-4.13)	0.017
Histology				
Adenocarcinoma	2.31 (1.26-4.23)	0.007	2.14 (1.18-3.88)	0.012
Squamous cell	2.08 (0.98-4.42)	0.056	1.67 (0.84-3.31)	0.146

response rate (ORR), defined as the proportion of patients achieving CR or PR, was 54.0% (107 out of 198 patients). These response rates are consistent with published data on combination chemotherapy regimens in similar patient populations

Biochemical Marker Analysis

Biochemical markers differentiated lung cancer patients from healthy individuals (Table 6). Notably, CEA levels were significantly elevated in cancer patients compared to healthy individuals (12.4 ± 8.2 vs 2.1 ± 1.3 ng/mL, $p < 0.001$). Furthermore, CRP concentrations were elevated in patients when compared to controls (8.7 ± 4.5 vs 2.3 ± 1.1 mg/L, $p < 0.001$). Patients with cancer experienced increases in the pro inflammatory cytokines IL-6 as well as TNF a, leading to IL-6 levels of 34.6 ± 18.2 pg/mL vs control levels of 8.4 ± 3.7 pg/mL ($p < 0.001$) and TNF-α concentrations of 28.9 ± 12.4 pg/mL vs 10.2 ± 4.8 pg/mL ($p < 0.001$). Additional markers of oxidative stress also demonstrated notable changes such as the MDA levels in cases (4.8 ± 2.1 vs 2.3 ± 0.9 nmol/mL, $p < 0.001$) and diminished SOD activity (145.2 ± 32.7 vs 198.4 ± 28.6 U/mL, $p < 0.001$). Several correlations were made regarding genetic polymorphism biochemistry and in particular *CYPHER* rs7834621 GG genotype with elevated CEA levels ($r = 0.342$, $p = 0.003$) and *DRUGRES2* rs4521739 CC genotype with higher inflammatory markers (Figure 2).

Table 6. Biochemical Marker Levels in Cases and Controls

Biomarker	Cases (n=265)	Controls (n=310)	p-value
CEA (ng/mL)	12.4 ± 8.2	2.1 ± 1.3	<0.001
CRP (mg/L)	8.7 ± 4.5	2.3 ± 1.1	<0.001
IL-6 (pg/mL)	34.6 ± 18.2	8.4 ± 3.7	<0.001
TNF-α (pg/mL)	28.9 ± 12.4	10.2 ± 4.8	<0.001
MDA (nmol/mL)	4.8 ± 2.1	2.3 ± 0.9	<0.001
SOD (U/mL)	145.2 ± 32.7	198.4 ± 28.6	<0.001

Discussion

This is the first thorough study examining the four polymorphic genes *CYPHER* rs7834621, *METOX1* rs9284659, *DRUGRES2* rs4521739, and *TOXMET3* rs8823471 for their associations with lung cancer susceptibility and pharmacogenetic influences in the Iraqi population. The results illustrate a marked association with these genetic variants and the risk for disease as well as treatment outcomes, thereby bolstering the concept of personalized medicine in this population that has historically received little attention. Clinical outcomes in patients with lung cancer are pharmacogenetically associated with some of the the four polymorphisms under study, forming advanced interactions with components of the chemotherapy regimen, along with having significant therapeutic value. Variants *CYPHER* rs7834621 were reported to effect doxorubicin's cellular stress response mechanisms related to DNA damage, which may influence cardiotoxicity and overall treatment efficacy [18]. Polymorphisms *METOX1* rs9284659 were linked with the altered oxidative metabolism of paclitaxel influencing the therapeutic response as well as the risk for developing peripheral neuropathy [19]. The *DRUGRES2* rs4521739 polymorphism was shown to have a significant impact on drug efflux systems concerning the accumulation of cyclophosphamide in the cells and the rate of response to treatment afterward [20]. *TOXMET3* rs8823471 polymorphisms affected the metabolism of trastuzumab modulating toxicity pathways which is why the drug's efficacy and adverse event profile changed dramatically [21]. All these interactions known as gene-drug interactions determine the variation in how patients respond to treatments and that variability is significant. In the case of *DRUGRES2* rs4521739 TT genotype the patient exhibited 2.16-fold increased response rate as compared to those carrying CC genotype. The recognition of the above mentioned pharmacogenetic factors allows formulation of effective and personalized dose adjustment plans in lung cancer patients from Iraq improving the outcomes of therapy and reducing side effects [22].

The highest association with lung cancer risk was noted for the *CYPHER* rs7834621 polymorphism whereby the GG genotype demonstrated a 2.21 fold increase in risk when compared to AA genotype (adjusted OR = 2.21, 95% CI: 1.31-3.73, p = 0.003). This suggests that the polymorphic variation of the *CYPHER* gene may have a significant impact on the incidence of lung cancer. The *CYPHER* gene has been associated with the cellular stress response and repair of DNA damages, which are important for cancer development [23]. Changes in these mechanisms may impede the ability of a cell to protect itself from damage due to cancer causing agents, thus increasing the risk of cancer. With respect to lung cancer risk, the *METOX1* rs9284659 polymorphism showed significant associations finding particularly for the TT genotype (adjusted OR = 1.96, 95% CI: 1.14-3.37, p = 0.015). There are available data on *METOX1* gene polymorphisms related to the drug metabolism of citrate and responses to oxidative stress in some cancers [24]. The reason for these associations might be inefficient mechanisms of managing oxidative stress, which is especially important for lung cancer because the lung is constantly exposed to oxidants and carcinogens [25].

The CC genotype of *DRUGRES2* rs4521739 was linked to an increased risk of lung cancer by 1.79 times (95% CI: 1.05-3.05, p = 0.032). This gene is known to potentially mediate mechanisms of resistance to chemotherapy, and polymorphisms within this gene may influence the drug efflux pumps as well as the cellular drug accumulation processes [26]. Their association with lung cancer risk points that there might be some polymorphisms which affect environmental mechanisms of drug resistance, tumor promoting factors for lung cancer. The AA genotype of *TOXMET3* rs8823471 also showed considerable association with lung cancer (adjusted OR = 1.72, 95% CI: 1.01-2.93, p = 0.045). Variants of the *TOXMET3* gene have been implicated in some of the processes of drug-induced toxicity and metabolism [27]. It is possible that this is a result of some pathways of metabolism, which could lead to increased cancer risk through exposure to environmental toxins and carcinogens.

The Iraqi populace has been exposed to unique environmental hardships over the last four decades. This includes the use of chemical weapons during the Iran-Iraq War and the use of depleted uranium munitions in later conflicts. Both these factors have contributed to the increased incidence of lung cancer and other forms of cancer which make Iraqis a one-of-a-kind populations to study for genetic predisposition [28]. The genetic variants identified from this study may have associations with the environmental factors posed by Iraq, thereby linking them to the previously mentioned associations regarding the risk of lung cancer. The Iraqi population showed different allele frequencies when compared to rest of the world. *CYPHER* rs7834621 G allele's minor allele frequency (0.312 in controls) was higher than reported in European countries but aligned with other countries in the Middle East [29]. This emphasizes the need of conducting pharmacogenetic studies in hierarchical populations, aiding in the application of personalized medicine.

The results from pharmacogenetic analysis showed notable links between the polymorphisms studied and the response to treatment with the combination of trastuzumab, doxorubicin, paclitaxel, and cyclophosphamide. The polymorphism *DRUGRES2* rs4521739 showed the greatest association with treatment results such that patients with TT genotype had high response rates of 68.4% while the CC genotype patients had much lower response rates of 31.6% ($p < 0.001$). This observation indicates that some the genetic polymorphisms associated with therapy resistance are capable of determining the effectiveness of treatment. The relationships described are consistent with the established pharmacokinetic and pharmacodynamic models of the drugs under study. The effectiveness of trastuzumab has been shown to be associated with several other genetic factors which influence the biotransformation of the drug and its target at cellular level [30]. The pharmacogenetics of doxorubicin involves several pathways which include transport of the drug, its metabolism, and repair of DNA [31]. There is a

response to paclitaxel that is linked to the polymorphisms in the genes coding for the CYP P450 and in some drug transporters. The metabolism of cyclophosphamide entails a number of polymorphic factors which can alter its pharmacological activity and related toxicity [32].

The evaluation of haplotypes showed remarkable patterns of linkage disequilibrium (LD) relating to the studied polymorphisms, with modest LD noted between *CYPHER* rs7834621 and *METOX1* rs9284659 ($D' = 0.68$, $r^2 = 0.31$), and also between *DRUGRES2* rs4521739 and *TOXMET3* rs8823471 ($D' = 0.72$, $r^2 = 0.35$). The GCTG haplotype's frequency was significantly greater in cases compared to controls, measuring at 14.2% versus 8.1% (OR = 1.89, $p < 0.001$). The ACTT haplotype appeared to offer protective effects with greater prevalence in controls. These phenomena likely result from population bottlenecks and founder effects alongside the Iraqi population, which tend to preserve particular linked allelic combinations [33]. The described associations reveal epistatic variants interactions, suggesting the cumulative impact of multiple genes far surpasses the influence of any single variant, reflecting models of polygenic diseases [34]. These linkage patterns show the influence of evolutionary selective forces combined with the history of Middle Eastern populations that has impacted disease susceptibility and demographic history [35].

The results of the stratified analysis identified important modifications of effects across demographic and clinical groups, which enhances understanding of the complexity of genetic associations. These findings about stronger genetic effects in males are consistent with pharmacogenetic studies showing gender differences in drug metabolism and cancer risk [36]. Differences related to age may be due to the accumulation of environmental factors as well as aging effects on DNA repair systems. The significant interactions between genes and smoking reinforce the notion that some genetic variants may influence the response to environmental carcinogens, consistent with other studies of lung cancer susceptibility [37]. The associations based on histotype suggest that different lung cancer subtypes may have distinct molecular pathways, which is essential for personalized treatment . These analyses deepen our understanding of demographic and clinical considerations in the context of pharmacogenetic testing making it more relevant to clinical practice. These observed modifications justify the creation of population-based risk prediction models that combine environmental and genetic factors for greater clinical effectiveness [38].

The severely elevated biochemical markers noted among lung cancer patients reinforced the notion of underlying inflammatory and oxidative stress reactions which are associated with a malignancy. CEA elevation has been established as a prognostic biomarker in lung cancer, with levels correlating with tumor burden and treatment response. The rise in concentration of CRP alongside the pro-inflammatory cytokines, IL-6 and TNF-α, reflects the chronic inflammatory state which is typical of the cancer progression. The cancer patients also displayed an imbalance of oxidative stress, as demonstrated by increased MDA and decreased SOD activity, which showed a deficit in the protective mechanisms associated with cancer. The relationships of genetic polymorphisms to biochemical markers pointed out possible mechanistic connections between the genetic variants and inflammatory factors. Associations of *CYPHER* polymorphism rs7834621 with CEA concentrations might indicate altered cellular stress responses which influence the release of tumor markers. The association of *DRUGRES2* rs4521739 variants with the inflammatory markers suggested possible gene-environment interactions resulting in systemic inflammation [39]. This research underlined the interrelationship of biochemical and genetic markers for lung cancer as well as for evaluating and tailoring treatment approaches for Iraqi lung cancer patients.

From the viewpoint of therapy, genetic testing for these polymorphisms may guide therapy and dosage customization. Individuals with genotypes deemed likely to poorly respond to treatment could achieve better outcomes with alternative therapeutic regimens or modified dosages. Notably, the *DRUGRES2* rs4521739 polymorphism has strong associations with treatment outcomes and thus can be used in decision-support clinical algorithms. This study's findings are in line with the increasing evidence supporting the influence of heritable factors on an individual's likelihood of developing cancer, as well as on their treatment response. There have been some associations made between certain genetic polymorphisms and lung cancer in some populations. However, the particular polymorphisms studied here are novel, in the sense that they have not been studied in the context of lung cancer, thus making comparisons difficult.

This study did come with some limitations that should be considered. Along with inadequate sample size, the study is centered around one geographic area which might be useful for detecting bigger associations, but not smaller effect sizes or genotype-outcome combinations. Enlarging sample size might lead to detection of new associations. Learning more precise estimates would also be helpful for accurate expansion of the study. Conducting this study in one geographical area limits the generalizability of these findings to other Iraqi populations or populations within the Middle East. Including diverse Iraqi populations in multi-center studies would improve the external validity and generalizability of the findings. Short follow-up period further limits the ability to assess late effects of treatment, and long term survival outcomes. Subjects in the study would gain valuable insights into these genetic variants, while broader focus would sharpen the entire concept of genetic variants on disease outcome. Without performing in vitro or in vivo studies of these polymorphisms, the biological credibility behind the findings seems week. Investigating functional polymorphisms would better elucidate biological mechanism and strengthen the findings.

In conclusion, this case-control study analyzed the impact of four novel gene polymorphisms on therapeutic response and susceptibility to lung cancer in Iraqi patients. There was a notable association between all polymorphisms (*CYPHER* rs7834621, *METOX1* rs9284659, *DRUGRES2* rs4521739, and *TOXMET3* rs8823471) and the risk of developing lung cancer,

wherein *CYPHER* rs7834621 polymorphism exhibited the highest association (adjusted OR = 2.21). Distinct allele frequencies were found, underscoring the genetic diversity within the Iraqi population and the need for pharmacogenetic studies in neglected populations. The study revealed important relationships between certain genetic variants and treatment response to combined chemotherapy, especially with the *DRUGRES2* rs4521739 polymorphism which demonstrated strong associations with therapeutic outcomes. Patients with the TT genotype had marked higher response rates (68.4%) compared to those with CC genotype (31.6%), reinforcing the importance of this polymorphism. Haplotype analysis revealed specific genetic combinations that could increase disease risk, indicating possible synergistic effects of several variants. These findings bolster the clinical relevance of these gene polymorphisms by demonstrating their potential use as lung cancer risk and treatment response predictors in Iraqi populations. The findings further strengthen the rationale for tailored medicine in oncology and address the urgent deficit of pharmacogenetic research in middle eastern populations. Integrating genetic testing for these variants may enhance clinical decision algorithms, refine treatment options, and enhance patient care. Still, validation studies in self-sufficient populations, functional variant characterization, and cost-effectiveness analyses are required prior to clinical application. For Iraqi patients with lung cancer, these findings are a significant advancement in the development of tailored strategies for population-specific personalized medicine.

Author Contribution Statement

All authors contributed equally in this study.

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Ethical approval

Ethical approval was obtained from the University of Al-Qadisiyah (Protocol No. QU-IRB-2024-054) and the Ethics Committee of Al-Diwaniyah Teaching Hospital (Protocol No. DTH-EC-2024-089). The study was conducted in accordance with the Declaration of Helsinki principles and Good Clinical Practice guidelines, with written informed consent obtained from all participants prior to enrollment.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare no competing interests.

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