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Role of Nrf2 and NQO1 genetic variants in cardiometabolic diseases: an 8-year prospective study of genetic, dietary, biochemical, and oxidative stress markers in Iranian Kurdish Adults

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This study assessed the association between Nrf2 (rs6721961) and NQO1 (rs1800566) polymorphisms and cardiometabolic diseases (CMDs) in the Kurdish population of Iran. We analyzed 509 Kurdish individuals. Biochemical markers were measured using commercial kits, and genotypes were determined via PCR-RFLP. Dietary patterns were evaluated using the food frequency questionnaire. Associations were analyzed using linear/Cox regression, margins plots, and generalized structural equation modeling (GSEM). We found biochemical, oxidative stress, and anthropometric parameters were significantly elevated in individuals carrying the recessive genotypes of Nrf2 and NQO1. Cox regression analysis revealed that homozygous recessive genotypes of each or both polymorphisms were strongly associated with an increased risk of hypertension (HTN) over time (HR = 2.82; 95% CI: 1.4–5.3). Similarly, carriers of the Nrf2 GT (HR = 1.75; 95% CI: 1.0–3.2) and carriers of both Nrf2 GT and NQO1 CT (HR = 3.15; 95% CI: 1.7–5.9) were significantly linked to a higher risk of type 2 diabetes mellitus (T2DM). According to GSEM analysis, Nrf2 GT and NQO1 (CT and TT) genotypes indirectly increased the risk of cardiovascular disease through inflammatory and oxidative stress pathways, fatty liver index, and dyslipidemia. This study demonstrates Nrf2 (rs6721961) and NQO1 (rs1800566) polymorphisms are significantly associated with an increased risk of CMDs, particularly HTN and T2DM, primarily through oxidative stress, inflammation, and metabolic dysregulation. These findings highlight the potential role of redox-related genetic variants in early risk prediction and personalized prevention strategies among genetically susceptible populations.

Keywords Cardiometabolic diseases, Oxidative stress, Inflammation, Metabolic dysregulation, Polymorphisms

Abbreviations

CMDs	Cardiometabolic diseases
GSEM	Generalized structural equation modeling
HTN	Hypertension
T2DM	Type 2 diabetes mellitus
CVD	Cardiovascular disease

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ROS	Reactive oxygen species
Nrf2	Nuclear factor E2-related factor 2
Keap1	Kelch-like ECH-associated protein 1
NQO1	NAD (P) H Quinone Dehydrogenase 1
SNPs	Single-nucleotide polymorphisms
Med Diet	Mediterranean diet
HEI	Healthy Eating Index
DII	Dietary Inflammation Index
FBS	Fasting blood sugar
TG	Triglycerides
TC	Total cholesterol
LDL-C	Low-density lipoprotein cholesterol
HDL-C	High-density lipoprotein cholesterol
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
GGT	Gamma-glutamyl transferase
Cr	Creatinine
GFR	Glomerular filtration rate
CRP	C-reactive protein
MDA	Malondialdehyde
TAC	Total antioxidant capacity
TOS	Total oxidative status
IOS	Inflammatory and oxidative stress
LFT	Liver function test
KFT	Kidney function test

Cardiometabolic diseases (CMDs), encompassing hypertension (HTN), type 2 diabetes mellitus (T2DM), and cardiovascular disease (CVD), include a complex network of interrelated metabolic disorders, including obesity, impaired lipid peroxidation, glucose intolerance, and vascular dysfunction, ultimately lead to high blood pressure and cardiovascular complications such as ischemic heart disease and heart failure[1,2].

Based on the last World Health Organization (WHO) reports, in 2022, 2.5 billion adults 18 years and older were overweight, with 890 million classified as obese[3]. The rising global obesity trend, observed in both high-income and developing nations, parallels an increase in CMDs prevalence, including HTN, T2DM, and CVD[4].

Iran, 2024 data reported an overweight/obesity prevalence of 18.38%[5]. A meta-analysis based on 53 studies showed that the prevalence of T2DM was significantly higher among Iranians aged 55–64 years (21.7%) compared to other age groups[6]. According to the data based on the American College of Cardiology/American Heart Association guidelines, the overall prevalence of CMDs risk in the Iranian population was reported as 26.26%, with the rates of 25.11% in women and 26.22% in men[7].

This growing burden of CMDs becomes a substantial global health concern, provoking extensive research aimed at deepening our understanding of the underlying pathology of these diseases[4].

CMDs are associated with disrupted redox homeostasis and elevated oxidative stress[4], characterized by increased reactive oxygen species (ROS) and reactive nitrogen species (RNS) that damage lipids, proteins, and DNA, impairing cellular function[8].

Growing evidence indicates that cells typically counteract the harmful effects of ROS/RNS by activating nuclear factor E2-related factor 2 (Nrf2; gene name Nfe2l2). Nrf2 is a transcription factor containing a basic leucine zipper (b-ZIP) domain. Under normal physiological conditions, its activity is suppressed by Kelch-like ECH-associated protein 1 (Keap1), which acts as a negative regulator[9].

During oxidative stress (OS), Keap1 undergoes oxidation and loses its inhibitory activity, allowing Nrf2 to translocate from the cytoplasm into the nucleus. Nrf2 binds to the antioxidant response element (ARE) upon entering the nucleus. It promotes the expression of various protective genes, including Heme Oxygenase 1 (HO-1), NAD (P)H Quinone Dehydrogenase 1 (NQO1), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT)[10].

The Nrf2 (rs6721961) variant in the promoter region diminishes the binding of transcription factors and ARE-related sites, leading to decreased gene transcription[11]. NQO1 (rs1800566) is a missense mutation in exon 6 that can directly affect transcription and produce an unstable protein with very low catalytic activity, which leads to a decrease in the capacity of the cell to detoxify quinones[12].

Thus, Nrf2 and NQO1 represent a promising target for developing new therapeutic agents that offer protection against CMDs by mitigating oxidative stress. In general, single-nucleotide polymorphisms (SNPs) in the Nrf2 gene and genes encoding related enzymes, such as NQO1, can alter the activation of Nrf2 signaling. This disruption may weaken the natural antioxidant defenses, leading to chronic inflammation and other harmful consequences[8].

Several studies investigated the association between the Nrf2 (rs6721961) and NQO1 (rs1800566) polymorphisms and CMDs[13–16]. Both polymorphisms result in reduced protein activity, which weakens the cellular antioxidant defense system against ROS[8,17].

Since any population could be characterized by unique genetic and environmental factors, studies on genetic polymorphisms may find different susceptibility to CMDs due to genetic, lifestyle, and also dietary patterns.

Research on various dietary patterns indicates that the Mediterranean diet (Med Diet) is linked to a considerable decrease in inflammation, especially systemic inflammation, in adults[18]. A 2025 study by Liweleya

et al. advocates for the Med Diet as a primary intervention for the prevention of HTN and CVD[19]. The outcomes of randomized controlled trials revealed the association between dietary compounds, such as polyphenols, monounsaturated fats, and omega-3 PUFAs, and the regulation of oxidative stress mechanisms through related signaling pathways involving NOX, NF-κB, and Nrf2, which may lead to decreased lipid oxidation and cardiovascular health improvements[20].

Due to the global increase in CMDs, research on genetic risk factors within specific populations, such as Iranian Kurds, is limited. Additionally, there is a necessity to investigate lifestyle factors and significant nutritional patterns to develop personalized disease preventive strategies customized to various ethnic groups. This research aims to address this gap by examining the correlation between Nrf2 (rs6721961) and NQO1 (rs1800566) polymorphisms and the development of CMDs in a cohort of 509 Iranian Kurds over 8 years by considering various underlying risk factors, including laboratory profiles, adherence to the Med Diet, and lifestyle.

Materials and methods

Study design & population

The current study includes 509 participants, 225 males and 284 females. All participants were recruited from the Ravansar Noncommunicable Disease (RaNCD) cohort. The RaNCD cohort study is one of 19 cohort sites participating in the Prospective Epidemiological Research Studies of Iranian Adults (PERSIAN) cohort, which focuses on individuals aged 35 to 65 who are permanent residents in various cohort sites. Ravansar County is located in the western part of Kermanshah province, Iran. All exclusive RaNCD information and the details of the PERSIAN cohort study can be found at http://persiancohort.com and in a previously published document profile[21]. The research ethics committee of Kermanshah University of Medical Sciences approved this study (Ethics code: IR.KUMS.MED.REC.1402.328).

Participants selection and data collection

This study utilized baseline data from the recruitment phase (between November 2014 and February 2017) of RaNCD. All participants were selected from those who were monitored from 2015 to 2023. In the recruitment phase, 10,047 individuals were included in the RaNCD. During the follow-up, 572 individuals were excluded due to death, migration, or withdrawal (Fig. 1). In total, 509 participants for the current study were chosen, including healthy subjects and those individuals who developed CMDs during the follow-up phase of the RaNCD cohort study. Cardiometabolic cases were detected based on the acceptable RaNCD follow-up pattern for diagnosing CMDs, as confirmed by the specific inclusion and exclusion criteria of the current study.

Follow-up methods

Active and passive yearly follow-up methods were used in the RaNCD cohort study. Data on incident outcomes (e.g., HTN, T2DM, and CVD) were collected via annual telephone interviews with all participants and self-reports from those visiting the cohort center.

Outcome assessment

Medical information (test results and hospitalization) related to people suspected of having T2DM, HTN, and CVD was referred to two specialists in collaboration with a general practitioner. In the event of a discrepancy in

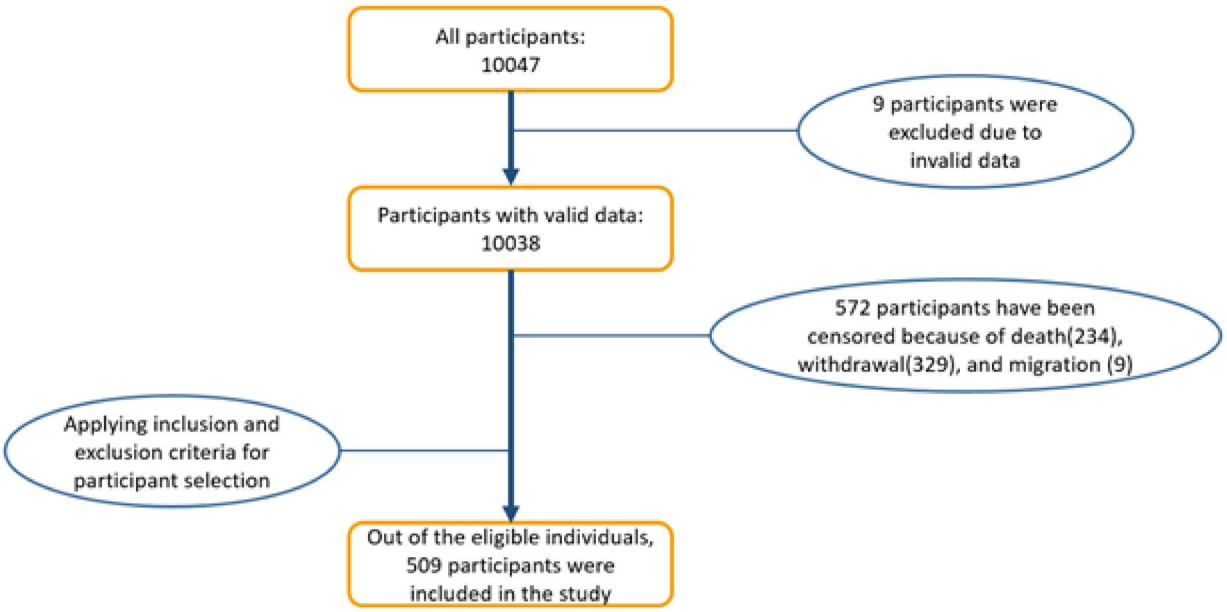


Fig. 1. Participant selection flowchart.

the diagnosis of the two specialists, it was referred to a third specialist for confirmation of the disease. Then, the confirmed information of the patients was recorded in the cohort database[21].

Diagnostic criteria

Hypertension was diagnosed per JNC-7 guidelines as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or use of antihypertensive drugs. T2DM was confirmed per ADA guidelines[22] with at least two of the following: fasting plasma glucose ≥ 126 mg/dL (7.0 mmol/L), HbA1c ≥ 6.5% (48 mmol/mol), 2-h plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during a 75-g oral glucose tolerance test, or use of antidiabetic medications.

Cardiovascular disease was diagnosed based on clinical records (e.g., ECG, imaging, and hospitalization) and specialist evaluation.

Study subjects (outcome and control groups)

Total number of participants diagnosed with HTN = 123 individuals.

Total number of participants diagnosed with T2DM = 119 individuals.

Total number of participants diagnosed with CVD = 118 individuals.

Control participants: 149 individuals.

The outcome groups were distinct, and no group had overlap; each group was analyzed independently in the statistical evaluations.

Exclusion & inclusion criteria

For the present investigation, we excluded individuals in the recruitment phase of RaNCD with cardiovascular diseases, diabetes (type 1 and 2), hypertension, non-alcoholic fatty liver disease (NAFLD), thyroid disorders, malignancies, inflammatory diseases such as rheumatoid arthritis, known lipid metabolism disorders, and autoimmune diseases.

Finally, 509 individuals were selected from among those who did not have the aforementioned medical conditions and continued to cooperate with the RaNCD study and were monitored annually.

Ethics approval and consent to participate

We confirm that all methods relevant to human participants were performed in accordance with the Declaration of Helsinki and approved by the research ethics committee of Kermanshah University of Medical Sciences (Ethics code: IR.KUMS.MED.REC.1402.328). The study protocol was fully explained to all participants, and each individual provided written informed consent to participate in the research.

Anthropometric indices measurements

Anthropometric indices were assessed using standardized protocols. Body weight was measured with a Bio Impedance Analyzer (InBody 770, BIOSPACE, Korea, accuracy ± 0.1 kg), height was measured using a stadiometer (accuracy ± 0.1 cm), and waist, hip, and wrist circumferences were measured with a calibrated anthropometric tape (accuracy ± 0.1 cm). Measurements were conducted by trained personnel under standardized conditions (fasting state), with duplicate measurements to ensure reliability. A comprehensive list of anthropometric indices (e.g., body mass index, waist-to-hip ratio) and CMDs indices (e.g., derived from anthropometric and biochemical data) and their *abbreviations* and *formulas* is provided in Supplementary Table S1.

Mediterranean diet score

The Med Diet was calculated using the Iranian Food Frequency Questionnaire (FFQ), assessing the consumption frequency and portion sizes of 118 food items[23,24]. Trained nutritionists conducted FFQ interviews, and nutrient intakes were analyzed using Nutritionist IV software (First Databank Inc., Hearst Corp., San Bruno, CA, USA). Participants with energy intakes exceeding 4,200 kcal/day (men) or 3,500 kcal/day (women) were excluded. The Med Diet score, based on Trichopoulou et al.[25] nine-point scale, evaluated nine components: vegetables, fruits, legumes, nuts, whole grains, seafood, meat, processed products, MUFA/SFA ratio, and alcohol (detailed in Supplementary Table S2). Components were scored 0 or 1 based on sex-specific median cut-offs, with intake (except alcohol) adjusted per 1,000 kcal. Healthy components scored 1 for intake ≥ median, unhealthy components scored 1 for intake < median, and moderate alcohol intake (men: 10–50 g/day; women: 5–25 g/day) scored 1. The total score ranges from 0 (low) to 9 (high adherence)[26]. Nutrition quality was assessed using the Healthy Eating Index-2015 (HEI-2015), scoring 0–100 across 13 food groups, with higher scores indicating better quality[27].

The Dietary Inflammation Index (DII) was calculated from FFQ data, considering over 45 food parameters (e.g., calories, saturated fat, fiber, omega-3 fatty acids, and flavonoids).

Lifestyle habits

Physical activity was measured using a validated 22-item questionnaire assessing work and leisure activities, expressed in METs (metabolic equivalents)-hours/day (6). The smoking status was evaluated using the National Health Insurance Scheme (NHIS)[21], and alcohol consumption was collected via self-report.

Biochemical measurements

To investigate the association between genetic polymorphisms and metabolic health, serum collected after an 8-hour fast was analyzed for biochemical markers using a Mindray BS-380 (China) auto-analyzer with commercial kits. Markers included fasting blood sugar (FBS), lipid profile (triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C)), liver

enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT)), and kidney function markers (urea, creatinine (Cr), glomerular filtration rate (GFR), uric acid, C-reactive protein (CRP)). Oxidative stress parameters (malondialdehyde (MDA), total antioxidant capacity (TAC), total oxidative status (TOS)) were measured via colorimetric assays. Hematological parameters (red blood cells (RBC), white blood cells (WBC), platelets (PLT)) were assessed using a Sysmex cell counter (Japan).

Genotyping

Genomic DNA was extracted from 2 mL of whole blood using the salting-out method[28]. The concentration and purity of the extracted DNA were evaluated using a NanoDrop 2000 spectrophotometer (Thermo Scientific). Genotyping for Nrf2 (rs6721961) and NQO1 (rs1800566) polymorphisms was performed via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The results were confirmed through gel electrophoresis. Details of primer sequences, PCR conditions, restriction enzyme protocols, and the images of agarose gel electrophoresis are provided in Supplementary Table S3 and Fig. S1.

Statistical analysis

Descriptive and comparative analyses

Categorical variables were summarized as numbers and percentages and analyzed using the Chi-square test. Hardy-Weinberg equilibrium (HWE) was tested to validate genotype distributions. Continuous variables were expressed as mean ± standard deviation (SD). For comparisons across multiple groups, one-way ANOVA was applied, followed by independent samples t-tests for pairwise comparisons (outcome groups vs. control; dominant vs. recessive genotypes).

Genotype stratification and comparative analysis

To enable detailed comparisons between individuals with different polymorphism combinations, genotypes were stratified into subgroups based on the presence or the absence of both Nrf2 (rs6721961) and NQO1 (rs1800566) polymorphisms (Table and Fig. 3).

Participants were classified into five mutually exclusive genotype groups:

Group 1 (Nrf2-GT) only included the carriers of the Nrf2 heterozygous genotype GT. Group 2 (NQO1-CT) only consisted of the carriers of the NQO1 heterozygous genotype CT. Group 3 (Homozygous Variants) were the Carriers of either Nrf2 homozygous variant TT, NQO1 homozygous variant TT, or both. Group 4 (Double Heterozygotes) consisted of carriers of both Nrf2 heterozygous (GT) and NQO1 heterozygous CT. Group 5 (Mixed Heterozygotes and Homozygotes) were the carriers of either Nrf2-GT + NQO1-TT or NQO1-CT + Nrf2-TT.

Survival and risk analysis

Time-to-event analysis was used Cox proportional hazards regression to evaluate CMDs risk over time. Results are presented as hazard ratios (HRs) with a 95% confidence interval (CI). Both unadjusted (crude) and adjusted models were evaluated. The adjusted models included five predefined covariates (as detailed in Table 3 footnotes).

Path analysis (GSEM)

Generalized Structural Equation Modeling (GSEM) was employed to assess the direct, indirect, and total effects of genotypes through the observed and latent variables.

Model construction

Confirmatory factor analysis was performed to validate latent variable structures. The best-fitting models were selected based on the following fit indices.

Comparative Fit Index (CFI) ≥ 0.95, Tucker-Lewis Index (TLI) ≥ 0.95, and Root Mean Square Error of Approximation (RMSEA)/Standardized Root Mean Square Residual (SRMR) < 0.08. Models for GSEM analysis were selected based on the lower Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC).

Model specifications

Binary outcomes were modeled using the Bernoulli distribution with a logit link function. Effects are reported as adjusted odds ratios (AORs). Final outputs included estimates of direct (Table 4), indirect, and total effects (Table 5), along with path diagrams illustrating observed and latent variables (Fig. 2).

Adjusted linear regression and marginal effects

Associations between genotypes and biochemical/anthropometric variables were assessed using multivariable linear regression, adjusting for age, gender, blood pressure, smoking status, dietary factors (HEI, DII, and Med Diet), and physical activity level. Results were visualized using margin plots (Fig. 3).

Sensitivity and robustness checks

Bootstrap analyses with 1,000 and 5,000 replications confirmed model stability, with results consistent with classical ordinary least squares (OLS) regression. Genotype distribution validity was further supported through HWE testing. Wide confidence intervals observed in specific estimates may come from limited sample size or high variability in the data, not model instability.

All statistical analyses were conducted using Stata version 18. A p-value < 0.05 was considered statistically significant.

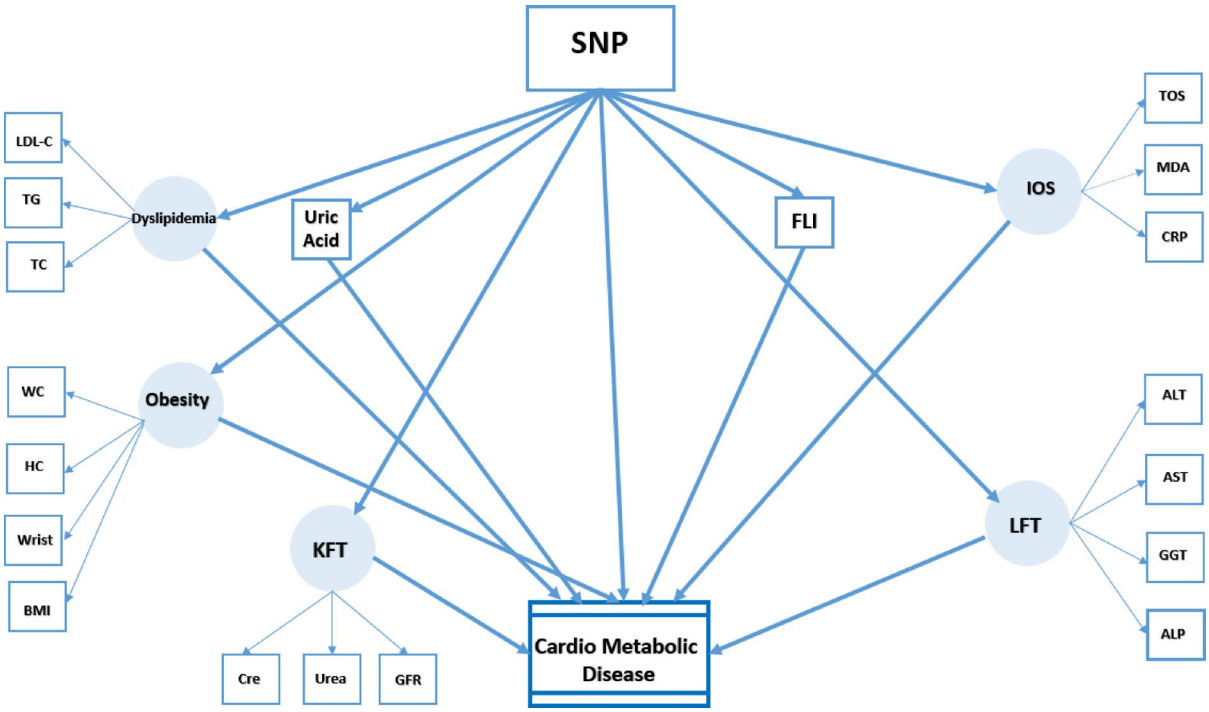


Fig. 2. Path diagram of the GSEM models showing the direct and indirect effects of Nrf2 and NQO1 (SNPs) on cardiometabolic diseases. The variables circled in blue represent latent variables derived from the observed variables (small squares). The FLI and uric acid are observed variables.

Results

Table 1 among 509 participants, there were 225 males, 44.2%, and 284 females, 55.8%. The number of females was 79 (53%) in controls, 74 females (60.2%) had HTN, 74 females (62.2%) had T2DM, and 66 females (55.9%) had CVD. The mean age of patients (excluding CVD: 51.05 ± 7.60 years) was similar to controls ($p > 0.05$). Passive smokers outnumbered current and former smokers. Of 93 menopausal females (32.7%), most had CVD (59.1%). Contraceptive use showed no differences.

HTN, T2DM, and CVD groups had higher SBP and DBP than controls, with the highest among HTN (SBP: 114.95 ± 18.00 mmHg; DBP: 74.58 ± 10.94 mmHg).

BMI and WC were higher in HTN, T2DM, and CVD patients ($p < 0.01$). HC and WHR were elevated in HTN and T2DM patients. T2DM patients had higher wrist circumference (17.50 ± 1.47 cm) and FFMI (18.51 ± 1.96). WWI, BAI, FMI, FLI, AIP, and CMI increased in disease groups. LAP was higher in females ($P < 0.001$).

FBS, TG, TC, and LDL-C levels were elevated in the disease groups, particularly in T2DM and CVD. ALP activity (204.76 ± 60.26 U/L) was higher in HTN, and GGT activity (25.38 ± 13.51 U/L) was higher in T2DM than in controls. Urea level (13.23 ± 3.12 mg/dL) increased in individuals with HTN, Cr (1.01 ± 0.21 mg/dL) in CVD, and uric acid in T2DM and CVD was elevated ($p < 0.05$). CRP, TOS, MDA, and OSI were higher in disease groups, while TAC (746.36 ± 162.61) was higher in controls ($p < 0.05$).

RBC and HCT were lower in CVD; WBC (7.01 ± 2.03 × 10^3 µL) and LMR were higher in HTN and CVD ($p < 0.05$) than in controls.

Based on the results of Table 2, no differences were found in age, gender, or smoking status between dominant and recessive genotype groups for Nrf2 and NQO1 ($P > 0.05$). Physical activity was lower in the NQO1 recessive group (39.75 ± 7.47; $P = 0.003$).

BMI and WC were higher in recessive genotype groups for Nrf2 ($P = 0.02$) and NQO1 ($P = 0.01$). HC (104.97 ± 9.61 cm, $P = 0.01$), WWI (11.53 ± 0.84, $P = 0.04$), and BAI (33.03 ± 6.38, $P = 0.03$) were elevated in the NQO1 recessive genotype. LAP was higher in female carriers of recessive genotypes (Nrf2: $P = 0.03$; NQO1: $P = 0.04$) and in males for the Nrf2 recessive group ($P = 0.02$). FMI was lower in dominant genotypes. AIP, CMI (Nrf2 recessive: 1.83 ± 1.35, $P = 0.01$), and FLI (NQO1 recessive: 88.91 ± 2.93, $P = 0.01$) were higher in individuals with recessive genotypes.

TG, TC, and LDL-C were elevated in carrier recessive genotypes (Nrf2 and NQO1), while HDL-C showed no difference. FBS was lower in dominant genotypes (Nrf2: 100.99 ± 32.06 mg/dL, $P = 0.04$). AST, GGT, urea, Cr, and uric acid were higher in recessive genotype groups.

CRP was higher in recessive carriers (Nrf2: $P = 0.02$; NQO1: $P = 0.06$). TOS, MDA, and OSI were elevated in recessive genotype groups ($P < 0.05$). In addition, recessive subjects had an elevated level of PLT and RDW ($P < 0.05$).

In Table 3, the results of Cox Regression Analysis for HTN, T2DM, and CVD Risk are presented. The genotype groups are detailed in Table 3 and in the statistical analysis section. In the crude analysis, Genotype

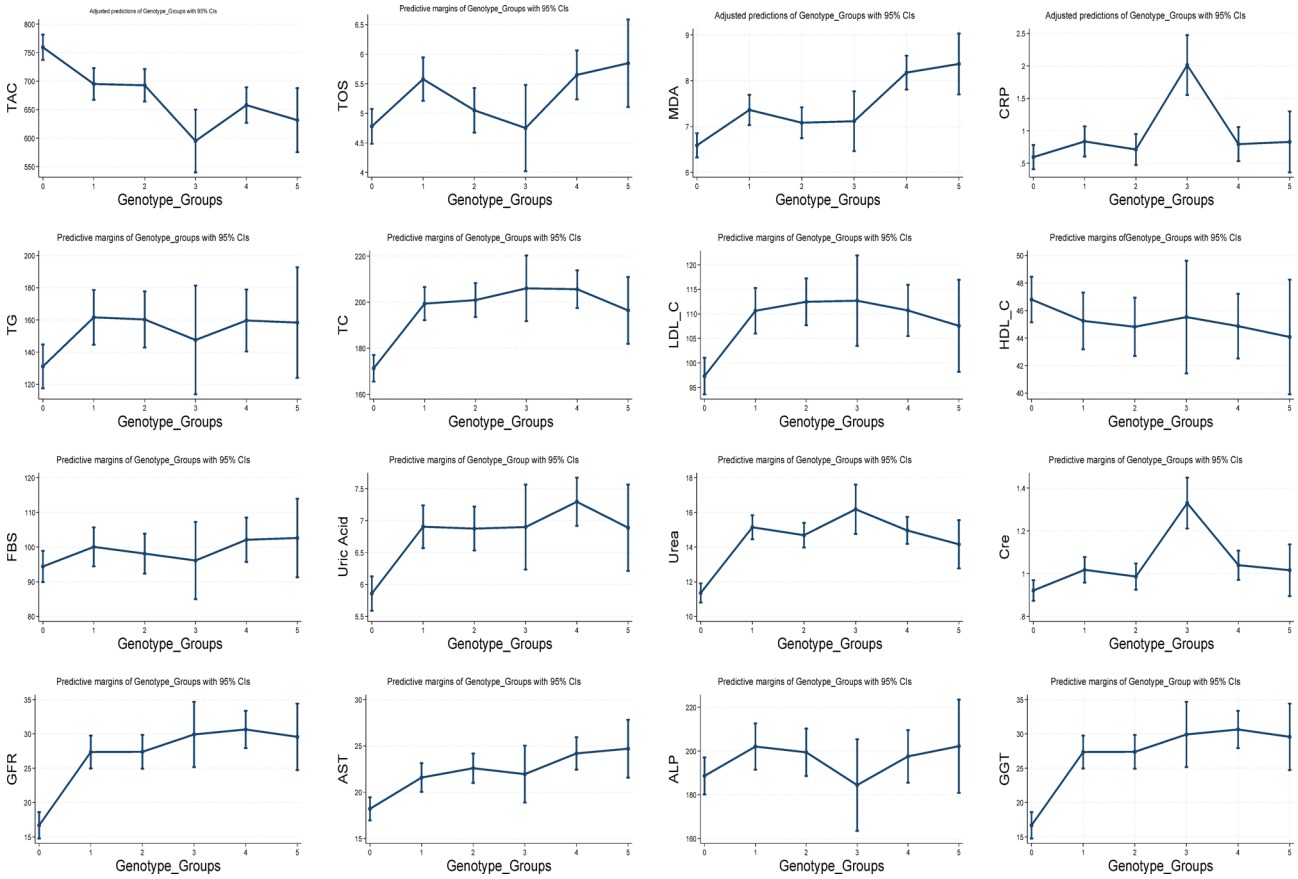


Fig. 3. Marginal effects plots of biochemical biomarkers by Nrf2 and NQO1 genotype Groups, derived from linear regression models adjusted for covariates.

Group 3 was significantly associated with an increased HTN risk (HR = 2.50, 95% CI: 1.4–4.3; Table 3). This association persisted across adjusted models 1–5, with the most substantial effect in model 4 (HR = 3.01, 95% CI: 1.5–5.5). Other genotype groups showed no significant associations.

Regarding T2DM, Genotype Groups 1, 4, and 5 were associated with increased T2DM risk in the crude analysis. Genotype Group 4 exhibited the strongest association in model 5 (HR = 3.15, 95% CI: 1.7–5.9), followed by Group 1 (HR = 2.14, 95% CI: 1.3–3.5). The association of Genotype Group 5 (HR = 1.99, 95% CI: 0.9–4.5) was not significant, as the CI included 1.0.

Moreover, we observed that no genotype groups were significantly associated with CVD risk.

Allele analysis revealed that carriers of the T allele of Nrf2 and NQO1 exhibited the highest risk for T2DM (Nrf2: HR = 2.19; 95% CI: 1.67–2.19; NQO1: HR = 1.66; 95% CI: 1.27–2.18) and HTN (Nrf2: HR = 1.43; 95% CI: 1.06–1.92; NQO1: HR = 1.46; 95% CI: 1.11–1.92). However, the associations were not statistically significant for CVD (Nrf2: HR = 1.23; 95% CI: 0.94–1.62; NQO1: HR = 0.91; 95% CI: 0.68–1.21).

Tables 4 and 5 present direct, indirect, and total effects of Nrf2 and NQO1 polymorphisms and CMDs risk factors on HTN, T2DM, and CVD via GSEM. We used the median ORs across models to provide a robust estimate for each genotype-outcome pair.

Table 4 (Direct effects)

The Nrf2 GT genotype was significantly associated with T2DM (OR = 2.82, 95% CI: 1.59–4.13) but not HTN or CVD. The Nrf2 TT genotype increased HTN risk (OR = 3.24, 95% CI: 1.37–7.60) but not T2DM or CVD. The NQO1 CT genotype increased HTN (OR = 1.46, 95% CI: 1.01–2.14) and T2DM risk (OR = 1.97, 95% CI: 1.28–3.01), while the TT genotype showed no significant effects.

The CMDs risk factors

Obesity increased T2DM (OR = 1.75, 95% CI: 1.36–2.27) and HTN risk. The latent inflammatory and oxidative stress (IOS) parameter was associated with all CMDs: HTN (OR = 2.30, 95% CI: 1.75–3.04), T2DM (OR = 2.36, 95% CI: 1.75–3.19), and CVD (OR = 3.07, 95% CI: 2.12–4.39). FLI and LFT increased the risk of T2DM. Uric acid increased CVD risk (OR = 1.17, 95% CI: 1.04–1.30). FBS, dyslipidemia, and kidney function test (KFT) showed no significant associations with disease.

Table 5 the GSEM analysis illustrates significant indirect and total effects (direct + indirect). These associations were analyzed through various metabolic and inflammatory pathways.

Variables	Controls $N=149$	Cardio-metabolic diseases			Total $N=509$	P-value[#]
		HTN $N=123$	T2DM $N=119$	CVD $N=118$		
Sex N (%)						
Female	70(47)	**74(60.2)***	**74(62.2)**	66(55.9)	284(55.8)	0.05
Male	79(53)	**49(39.8)**	**45(37.8)**	52(44.1)	225(44.2)	
Age	47.78 ± 4.89	48.08 ± 8.53	47.97 ± 7.85	**51.05 ± 7.60**	48.66 ± 7.34	< 0.001
Alcohol Use: Yes (%)	10(6.7)	3(2.4)	4(3.4)	5(4.25)	22(4.3)	0.3
Smoking status N (%)						
No-smoker	56(38.62)	55(45.08)	51(43.59)	42(35.90)	204(40.72)	0.01
Current-smoker	20(13.79)	7(5.74)	9(7.69)	26(22.22)	62(12.38)	
Former-smoker	11(7.59)	12(9.84)	7(5.98)	10(8.55)	40(7.98)	
Passive-smoker	58(40)	48(39.34)	50(42.74)	39(33.33)	195(38.92)	
Physical activity METs	41.60 ± 8.33	40.57 ± 8.03	40.35 ± 8.34	41.28 ± 9.82	40.98 ± 8.62	0.1
Menopause N (%)						
No	54(79.41)	53(71.62)	51(68.92)	**33 (48.5)**	191(67.26)	0.003
Yes	14(20.59)	21(28.38)	23 (31.08)	**35 (51.5)**	93 (32.74)	
Use a contraceptive drug						
No	8(11.76)	11(14.86)	15(17.57)	11(16.67)	43(15.25)	0.7
Yes	60(88.24)	63(85.14)	61(82.43)	55(83.33)	239(84.75)	
Med diet score	4.07 ± 1.62	3.78 ± 1.49	4.32 ± 1.61	3.81 ± 1.67	4 ± 1.61	0.6
DII	−1.94 ± 1.50	−2.29 ± 1.42	−2.03 ± 1.65	**−2.45 ± 1.50**	−2.16 ± 1.53	0.4
HEI	51.89 ± 6.84	51.79 ± 7.33	53.29 ± 7.04	50.86 ± 6.94	51.96 ± 7.06	0.8
Systolic blood pressure (mmHg)	101.84 ± 11.51	**114.95 ± 18**	**112.62 ± 14.57**	**113.35 ± 21.74**	110.2 ± 17.44	< 0.001
Diastolic blood pressure (mmHg)	66.75 ± 7.7	**74.58 ± 10.94**	**72.55 ± 9.65**	**72.53 ± 11.34**	71.34 ± 10.32	< 0.001
Anthropometric & cardiometabolic indices						
BMI(kg/m^2)	26.94 ± 4.27	**29.10 ± 4.40**	**30.56 ± 4.75**	**28.15 ± 4.49**	28.59 ± 4.65	< 0.001
WC(cm)	95.03 ± 10.58	**100.59 ± 11.01**	**102.87 ± 10.84**	**98.44 ± 10.29**	99.0 ± 11.05	0.8
HC (cm)	101.03 ± 8.35	**105.42 ± 9.49**	**106.53 ± 10.23**	103.05 ± 9.08	103.85 ± 9.49	0.1
WHR	0.93 ± 0.05	**0.96 ± 0.05**	**0.97 ± 0.06**	0.94 ± 0.06	0.95 ± 0.06	0.7
Wrist circumference(cm)	17.03 ± 1.39	17.3 ± 1.43	**17.5 ± 1.47**	17.28 ± 1.32	17.26 ± 1.41	0.6
VAI(male)	2.49 ± 2.3	2.55 ± 1.77	2.55 ± 1.55	2.34 ± 1.64	2.48 ± 1.90	0.008
VAI(female)	3.80 ± 3.52	3.88 ± 2.70	3.88 ± 2.43	3.57 ± 2.44	3.78 ± 2.90	0.008
ABSI	0.83 ± 0.05	0.83 ± 0.05	0.83 ± 0.04	0.84 ± 0.04	0.83 ± 0.05	0.2
WWI	11.2 ± 0.79	**11.5 ± 0.88**	**11.5 ± 0.88**	**11.5 ± 0.78**	11.44 ± 0.83	0.5
BAI	30.40 ± 5.79	**33.11 ± 6.15**	**34.03 ± 6.61**	**32.50 ± 5.86**	32.39 ± 6.23	0.4
LAP(male)	54.22 ± 47.21	63.94 ± 38.79	70.69 ± 40.96	57.09 ± 45.86	60.29 ± 44.12	0.4
LAP(female)	46.36 ± 28.79	**66.08 ± 30.24**	**85.89 ± 43.41**	**85.46 ± 58.9**	70.83 ± 44.48	< 0.001
Free fat mass index	17.88 ± 1.64	18.17 ± 1.81	**18.51 ± 1.96**	17.97 ± 1.79	18.12 ± 1.8	0.2
Fat mass index	9.05 ± 3.82	**10.93 ± 3.92**	**12.04 ± 4.22**	**10.17 ± 3.94**	10.47 ± 4.11	0.7
AIP	0.90 ± 0.71	**1.07 ± 0.59**	**1.23 ± 0.57**	**1.17 ± 0.66**	1.08 ± 0.65	0.04
CMI	1.49 ± 1.33	1.61 ± 1.10	**1.80 ± 1.01**	**1.85 ± 1.40**	1.67 ± 1.23	0.001
FLI	86.95 ± 5.02	**88.87 ± 3.21**	**89.81 ± 2.70**	**88.32 ± 4.03**		< 0.001
Laboratory test results						
FBS(mg/dl)	86.86 ± 9.59	**98.74 ± 34.32** $P=0.0001$	**106.79 ± 27.72** $P<0.001$	**102.90 ± 37.48** $P<0.001$	98.08 ± 29.51	< 0.001
TG(mg/dl)	132.01 ± 93.62	146.31 ± 73.76	**165.77 ± 75.85**	**166.14 ± 108.84**	151.24 ± 90.1	< 0.001
Chol (mg/dl)	183.95 ± 36.75	186.79 ± 33.80	**196.76 ± 37.25**	**200.40 ± 42.83**	191.42 ± 38.1	0.07
LDL-C (mg/dl)	101.05 ± 24.66	103.89 ± 20.78	**109.43 ± 24.78**	**112.57 ± 27.17**	106.36 ± 24.7	0.03
HDL-C (mg/dl)	46.38 ± 11.73	45.78 ± 10.56	44.91 ± 10.94	44.91 ± 11.38	45.56 ± 11.18	0.6
ALT(IU/L)	23.56 ± 11.09	24.70 ± 13.35	**30.67 ± 22.43**	22.52 ± 9.64	25.25 ± 15.03	< 0.001
AST(IU/L)	20.86 ± 6.97	20.43 ± 6.54	**24.06 ± 12.01**	20.16 ± 6.26	21.35 ± 8.33	< 0.001
ALP(IU/L)	185.77 ± 51.95	**204.76 ± 60.26**	**201.92 ± 54.11**	191.61 ± 54.38	195.49 ± 55.5	0.3
GGT(IU/L)	21.33 ± 12.32	24.28 ± 14.11	**29.76 ± 15.88**	**25.38 ± 13.51**	24.95 ± 14.22	0.03
Urea(mg/dl)	14.28 ± 4.46	**13.23 ± 3.12**	13.39 ± 4.01	14.24 ± 4.38	13.81 ± 4.06	< 0.001
Cre(mg/dl)	0.97 ± 0.14	0.96 ± 0.15	0.98 ± 0.19	**1.01 ± 0.21**	0.98 ± 0.17	< 0.001
GFR	76.10 ± 10.5	77.87 ± 6.17	75.90 ± 13.8	**65.24 ± 18.9**	74.34 ± 13.34	0.06
Uric Acid (mg/dl)	6.33 ± 1.64	6.61 ± 1.68	**6.74 ± 1.80**	**7.04 ± 2.12**	6.66 ± 1.82	0.01
Continued						

Variables	Controls $N=149$	Cardio-metabolic diseases			Total $N=509$	P-value#
		HTN $N=123$	T2DM $N=119$	CVD $N=118$		
CRP(mg/dL)	0.56 ± 0.44	**0.81 ± 0.73**	**0.70 ± 0.70**	**0.83 ± 0.31**	0.71 ± 0.58	< 0.001
TAC (nmol Trolox Eq./L)	746.36 ± 162.61	**685.93 ± 121.44**	**671.88 ± 132.99**	**698.91 ± 150.26**	19.53 ± 4.06	0.005
TOS(nmol/ml)	4.15 ± 3.06	**5.52 ± 0.65**	**5.43 ± 0.74**	**5.99 ± 1.09**	19.28 ± 7.16	< 0.001
OSI(TOS/TAC)	0.005 ± 0.004	**0.008 ± 0.008**	**0.008 ± 0.001**	**0.008 ± 0.001**	0.007 ± 0.005	< 0.001
MDA(nmol/ml)	4.56 ± 0.76	**8.16 ± 0.21**	**8.48 ± 0.52**	**8.39 ± 0.52**	1.34 ± 0.17	< 0.001
CBC test results						
RBC(10^6 µ/L)	5.05 ± 0.55	4.99 ± 0.61	4.93 ± 0.43	**4.88 ± 0.56**	4.97 ± 0.55	0.08
Hb (g/dl)	14.42 ± 1.56	14.23 ± 1.54	14.17 ± 1.44	14.09 ± 1.48	14.24 ± 1.51	0.8
HCT (%)	40.39 ± 4.05	39.78 ± 4.05	39.54 ± 3.58	**39.33 ± 3.65**	39.80 ± 3.86	0.3
MCV (fL)	80.32 ± 6.76	80.14 ± 7.41	80.32 ± 5.48	80.91 ± 6.49	80.41 ± 6.58	0.01
MCH (pg)	28.71 ± 2.94	28.7 ± 3.05	28.80 ± 2.40	29.02 ± 2.92	28.80 ± 2.84	0.05
MCHC (g/dl)	35.69 ± 1.43	35.78 ± 1.36	35.83 ± 1.31	35.82 ± 1.58	35.77 ± 1.42	0.2
RDW-CV (%)	11.11 ± 1.33	11.12 ± 1.21	11.05 ± 1.29	11.11 ± 1.25	11.10 ± 1.27	0.7
WBC (10^3 µ/L)	6.51 ± 1.73	**7.01 ± 2.03**	6.83 ± 1.51	6.54 ± 1.55	6.71 ± 1.73	0.004
GR%	54.99 ± 9.32	56.90 ± 10.16	55.33 ± 8.90	54.87 ± 8.81	55.51 ± 9.33	0.3
Lymph%	41.60 ± 8.76	39.57 ± 9.32	41.31 ± 8.26	41.57 ± 8.37	41.03 ± 8.71	0.5
Mono%	3.39 ± 1.22	3.48 ± 1.34	3.35 ± 1.22	3.54 ± 1.08	3.44 ± 1.22	0.1
PLT(10^3 µ/L)	258.55 ± 59.54	267.24 ± 64.67	270.87 ± 66.23	251.99 ± 57.39	262.01 ± 62.51	0.3
PDW (%)	16.80 ± 0.96	16.96 ± 0.75	16.87 ± 0.62	16.95 ± 0.71	16.89 ± 0.78	< 0.001
LHR	0.94 ± 0.29	0.91 ± 0.32	0.96 ± 0.27	0.98 ± 0.31	0.95 ± 0.30	0.2
MHR	0.07 ± 0.03	0.08 ± 0.03	0.07 ± 0.03	0.08 ± 0.03	0.07 ± 0.03	0.05
LMR	13.55 ± 4.83	**12.32 ± 3.82**	13.64 ± 4.79	**12.51 ± 3.90**	13.03 ± 4.41	0.007

Table 1. Demographic and laboratory characteristics of the study population. *Significant differences ($P<0.05$) between disease groups and controls, as determined by independent samples t-tests, are bolded. # The P-value of the one-way ANOVA test between groups. RBC Red blood cell; Hb Hemoglobin; HCT Hematocrit; MCV Mean corpuscular volume; MCH Mean Corpuscular Hemoglobin; MCHC Mean Corpuscular Hemoglobin Concentration; RDW Red cell distribution Width; WBC White blood cell count; GR% Granulocytes percentage; Lymph% lymphocytes percentage; Mono% Monocytes percentage; PLT: Platelet count; PDW Plateletldth. LHR Lymphocyte% to HDL-C ratio; MHR Monocyte% to HDL-C ratio; LMR Lymphocyte% to Monocyte% ratio.

Nrf2 genotypes

Nrf2 GT genotype had the strongest total effect for T2DM (OR = 4.94, 95% CI: 1.29–3.16). This association was driven by both direct and indirect effects via obesity, FBS, OSI, FLI, and LFT. The Nrf2 TT genotype total effect for HTN was positive, and significantly increased the risk of HTN (OR = 5.11, 95% CI: 1.94–13.4) via IOS. In addition, through other pathways (Table 5), the total risk of HTN overall increased by over 3-fold.

NQO1 genotypes

The NQO1 CT genotype had a significant total effect on the HTN risk. Nevertheless, only IOS indirectly enhanced the HTN risk (OR = 1.26, 95%CI: 1.07–1.48). The highest indirect and total effect of the NQO1 CT genotype was for T2DM risk, particularly via IOS (OR = 2.12, 95% CI: 1.15–3.92) and LFT (OR = 1.97, 95%CI: 1.28–3.01) paths. The NQO1 TT genotype via the OSI path increased the risk of CMDs.

Our GSEM models identified several key mediators significantly contributing to the development of CMDs. The IOS was the most potent mediator across all genotypes. The NQO1 TT carriers via the IOS path significantly increased the CVD risk. FLI & LFT could mediate the effects of NQO1 variants in HTN and T2DM diseases. For CVD risk, uric acid emerged as a key mediator in the overall model.

Based on the results, the Nrf2 GT genotype acts primarily through metabolic pathways (obesity, FBS) to increase T2DM risk, and the Nrf2 TT genotype had a substantial direct effect on HTN, may suggest a more independent genetic mechanism rather than mediation by metabolic or inflammatory factors. Also, both CT and TT genotypes of NQO1 contribute to HTN and T2DM through a combination of direct and indirect mechanisms; their effects are strongly modulated by IOS and liver dysfunction, which could indicate a central role of redox homeostasis and metabolic dysregulation.

Furthermore, we evaluated the proportion of mediation on the path to CMDs; Nrf2 GT increased T2DM risk, with ~ 50% mediated via OSI. Nrf2 TT increased HTN risk, with ~ 30% mediated via obesity and dyslipidemia. NQO1 CT enhanced HTN and T2DM risk, with 40–60% mediated via OSI and FLI. NQO1 TT showed no significant association with HTN or T2DM (< 50% mediation), indicating a weak genetic effect.

Figure 3 and Supplementary Fig. S2 demonstrate the margin plots derived from the linear regression analysis between genotype groups (mentioned in the statistical section) and various biochemical and anthropometric parameters after adjusting for confounders, respectively. The predictive margins with 95% confidence intervals

Variables	Nrf2 genotypes		*P*-value	NQO1 genotype		*P*-value
	Dominant (GG)	Recessive (GT + TT)		Dominant (CC)	Recessive (CT + TT)	
Sex N (%)						
Female	127(45.2)	98(43)	0.6	127(45.2)	98(43)	0.6
Male	154(54.8)	130(57)		154(54.8)	130(57)	
Age	48.72 ± 7.35	48.57 ± 7.34	0.8	48.92 ± 7.36	48.33 ± 7.31	0.3
Alcohol use: Yes (%)	14(4.98)	8(3.51)	0.4	17(6.05)	5(2.19)	**0.03**
Smoking status N (%)						
No-smoker	120(43.48)	84(37.33)	0.3	108(38.99)	96(42.86)	0.6
Current-smoker	31(11.23)	31(13.78)		38(13.72)	24(10.71)	
Former-smoker	24(8.70)	16(7.11)		24(8.66)	16(7.14)	
Passive-smoker	101(36.59)	94(41.78)		107(38.63)	88(39.29)	
Physical activity METs	40.89 ± 8.29	41.10 ± 9.03	0.7	41.99 ± 9.35	39.75 ± 7.47	**0.003**
Menopause N (%)						
Yes	51(33.5)	40(30.8)	0.6	55(36.2)	36(27.7)	0.1
No	101(66.5)	90(69.2)		97(63.8)	94(72.3)	
Use a contraceptive drug						
Yes	130(85.5)	109(83.8)	0.6	123(80.9)	116(89.2)	0.06
No	22(14.5)	21(16.2)		29(19.1)	14(10.8)	
Med lcore	3.93 ± 1.53	4.07 ± 1.70	0.3	4.02 ± 1.61	3.97 ± 1.61	0.7
DII	−2.24 ± 1.50	−2.07 ± 1.56	0.2	−2.13 ± 1.52	−2.21 ± 1.53	0.5
HEI	51.42 ± 6.95	52.63 ± 7.15	0.05	51.60 ± 6.91	52.40 ± 7.23	0.2
Systolic blood pressure (mmHg)	110.23 ± 18.02	110.15 ± 16.74	0.9	110.72 ± 18.88	109.54 ± 15.50	0.4
Diastolic blood pressure (mmHg)	71.34 ± 10.24	71.34 ± 10.44	0.9	71.47 ± 10.77	71.18 ± 9.75	0.7
Anthropometric & cardiometabolic indices						
BMI(kg/m^2)	28.21 ± 4.62	29.07 ± 4.66	**0.03**	28.16 ± 4.70	29.12 ± 4.55	**0.02**
WC(cm)	98.12 ± 11.33	100.09 ± 10.62	**0.04**	97.71 ± 10.98	100.59 ± 10.95	**0.003**
HC (cm)	103.19 ± 9.25	104.66 ± 9.73	0.08	102.93 ± 9.30	104.97 ± 9.61	**0.01**
WHR	0.95 ± 0.06	0.95 ± 0.06	0.3	0.95 ± 0.06	0.96 ± 0.06	0.07
Wrist circumference(cm)	17.16 ± 1.43	17.39 ± 1.38	0.06	17.23 ± 1.43	17.31 ± 1.39	0.5
VAI(male)	2.27 ± 1.57	2.76 ± 2.24	0.06	2.36 ± 2.03	2.63 ± 1.73	0.2
VAI(female)	3.46 ± 2.40	4.20 ± 3.42	0.06	3.60 ± 3.09	4.01 ± 2.63	0.2
ABSI	0.83 ± 0.05	0.83 ± 0.05	0.8	0.83 ± 0.05	0.83 ± 0.05	0.3
WWI	11.42 ± 0.85	11.47 ± 0.80	0.4	11.38 ± 0.81	11.53 ± 0.84	**0.04**
BAI	32.07 ± 6.16	32.77 ± 6.32	0.2	31.86 ± 6.07	33.03 ± 6.38	**0.03**
LAP(male)	55.31 ± 39.04	66.75 ± 49.41	**0.04**	58.93 ± 47.61	62.06 ± 39.32	0.5
LAP(female)	64.16 ± 39.97	78.79 ± 48.28	**0.005**	65.16 ± 45.34	77.60 ± 42.62	**0.01**
Free mass index	18.02 ± 1.81	18.24 ± 1.79	0.1	18.09 ± 1.86	18.16 ± 1.73	0.6
Fat mass index	10.18 ± 4.02	10.83 ± 4.20	0.07	10.07 ± 4.07	10.96 ± 4.11	**0.01**
AIP	1.01 ± 0.64	1.17 ± 0.66	**0.005**	1.02 ± 0.67	1.15 ± 0.61	**0.01**
CMI	1.55 ± 1.12	1.83 ± 1.35	**0.01**	1.61 ± 1.26	1.76 ± 1.19	0.1
FLI	88.14 ± 4.34	88.73 ± 3.63	0.1	87.99 ± 4.73	88.91 ± 2.93	**0.01**
Laboratory test results						
FBS(mg/dl)	95.75 ± 27.12	100.99 ± 32.06	**0.04**	97.04 ± 28.57	99.38 ± 30.65	0.3
TG(mg/dl)	141.73 ± 85.0	163.01 ± 95.09	**0.008**	145.35 ± 92.25	158.54 ± 87.20	0.1
Chol(mg/dl)	183.66 ± 39.45	201.07 ± 34.27	**< 0.001**	182.89 ± 39.60	202.02 ± 33.55	**< 0.001**
LDL-C (mg/dl)	103.43 ± 26.53	109.98 ± 21.99	**0.003**	102.59 ± 24.66	111.01 ± 24.20	**0.0001**
HDL-C (mg/dl)	46.12 ± 11.34	44.85 ± 10.96	0.2	45.99 ± 11.64	45.02 ± 10.57	0.3
ALT(IU/L)	24.43 ± 15.08	26.28 ± 14.95	0.1	24.23 ± 14.64	26.53 ± 15.45	0.08
AST(IU/L)	20.10 ± 7.34	22.88 ± 9.19	**0.0002**	19.64 ± 7.35	23.46 ± 8.98	**< 0.001**
ALP(IU/L)	192.72 ± 53.47	198.92 ± 57.89	0.2	193.67 ± 56.64	197.75 ± 54.12	0.4
GGT(IU/L)	21.17 ± 12.92	29.61 ± 14.39	**< 0.001**	21.37 ± 12.87	29.37 ± 14.58	**< 0.001**
Urea(mg/dl)	12.81 ± 3.72	15.04 ± 4.14	**< 0.001**	12.99 ± 4.07	14.82 ± 3.83	**< 0.001**
Cre(mg/dl)	0.95 ± 0.15	1.02 ± 0.18	**< 0.001**	0.96 ± 0.15	1.01 ± 0.19	**0.001**
GFR	74.53 ± 10.7	73.97 ± 17.5	0.8	75.6 ± 12.89	72.83 ± 13.97	0.4
Uric acid (mg/dl)	6.31 ± 1.66	7.09 ± 1.91	**< 0.001**	6.30 ± 1.81	7.09 ± 1.74	**< 0.001**
CRP(mg/dL)	0.63 ± 0.72	0.82 ± 0.29	**0.0004**	0.69 ± 0.52	0.74 ± 0.64	0.3
Continued						

Variables	Nrf2 genotypes			NQO1 genotype		
	Dominant (GG)	Recessive (GT + TT)	*P*-value	Dominant (CC)	Recessive (CT + TT)	*P*-value
TAC (nmol Trolox Eq./L)	730.33 ± 160.22	664.35 ± 134.35	**< 0.001**	729.81 ± 166.52	664.99 ± 124.89	**< 0.001**
TOS(nmol/ml)	4.88 ± 2.06	5.57 ± 1.75	**0.0001**	5.10 ± 2.28	5.30 ± 1.46	0.2
MDA(nmol/ml)	6.77 ± 1.88	7.78 ± 1.55	**< 0.001**	6.93 ± 1.83	7.59 ± 1.73	**< 0.001**
CBC test results						
RBC(10^6 µ/L)	4.99 ± 0.54	4.95 ± 0.56	0.4	4.94 ± 0.54	5.0 ± 0.56	0.2
Hb (g/dl)	14.32 ± 1.52	14.13 ± 1.49	0.1	14.20 ± 1.53	14.28 ± 1.49	0.5
HCT (%)	40.01 ± 3.92	39.55 ± 3.78	0.1	39.61 ± 3.88	40.04 ± 3.83	0.2
MCV (fL)	80.53 ± 6.52	80.26 ± 6.66	0.6	80.46 ± 6.71	80.35 ± 6.42	0.8
MCH (pg)	28.87 ± 2.83	28.71 ± 2.86	0.5	28.89 ± 2.93	28.69 ± 2.72	0.4
MCHC (g/dl)	35.80 ± 1.37	35.74 ± 1.48	0.5	35.85 ± 1.47	35.67 ± 1.35	0.1
RDW-CV (%)	11.01 ± 0.99	11.31 ± 1.21	**0.002**	10.99 ± 1.03	11.34 ± 1.16	**0.0003**
WBC (10^3 µ/L)	6.60 ± 1.70	6.85 ± 1.76	0.1	6.71 ± 1.88	6.72 ± 1.53	0.9
GR%	55.43 ± 9.09	55.60 ± 9.64	0.8	55.60 ± 9.75	55.39 ± 8.80	0.8
Lymph%	41.11 ± 8.56	40.94 ± 8.92	0.8	40.97 ± 9.10	41.12 ± 8.22	0.8
Mono%	3.44 ± 1.17	3.43 ± 1.28	0.9	3.41 ± 1.21	3.47 ± 1.23	0.5
PLT(10^3 µ/L)	252.56 ± 60.79	273.72 ± 61.95	**0.0001**	252.93 ± 63.03	273.26 ± 59.27	**0.0002**
PDW (%)	16.93 ± 0.67	16.84 ± 0.90	0.2	16.91 ± 0.84	16.87 ± 0.71	0.5
LHR	0.94 ± 0.28	0.96 ± 0.32	0.2	0.94 ± 0.30	0.96 ± 0.30	0.5
MHR	0.07 ± 0.03	0.08 ± 0.03	0.4	0.07 ± 0.03	0.08 ± 0.03	0.3
LMR	13.08 ± 4.52	12.97 ± 4.29	0.7	12.99 ± 4.22	13.08 ± 4.65	0.8

Table 2. Demographic and laboratory characteristics by Nrf2 and NQO1 genotypes (dominant vs. recessive models). * Significant results ($P < 0.05$) between dominant and recessive groups, as determined by independent samples t-tests, are bolded. RBC Red Blood Cell; Hb Hemoglobin; HCT Hematocrit; MCV Mean Corpuscular Volume; MCH Mean Corpuscular Hemoglobin; MCHC Mean Corpuscular Hemoglobin Concentration; RDW Red Cell Distribution Width; WBC White Blood Cell count; GR% Granulocytes Percentage; Lymph% Lymphocytes Percentage; Mono% Monocytes Percentage; PLT Platelet count; PDW Platelet Distribution Width. LHR Lymphocyte% to HDL-C ratio; MHR Monocyte% to HDL-C ratio; LMR Lymphocyte% to Monocyte% ratio.

(CIs) are presented for each outcome variable across different genotype groups (1–5). Based on the margins plot, Genotype Groups 4 and 5 are associated with an elevated level of oxidative stress markers (TOS, MDA); the highest level of CRP and unfavorable lipid profiles (higher TC and LDL-C), and kidney function test (higher Cr and urea) was seen in Genotype Group 3 compared to the other genotype groups.

Genotype Group 4 is associated with the most adverse outcomes across multiple biomarkers, including elevated FBS, Uric Acid, Urea, impaired renal function (low GFR), and increased liver enzyme activities (AST, GGT), and exhibited higher levels of HC, WC, wrist circumference, WWI, and FFMI, compared with other groups. A pattern for adipose composition and anthropometric measurements like BMI, FMI, and AIP was observed from Genotype Group 5. Regarding anthropometric indices, genotype Group 3 did not considerably increased the parameters compared to genotype Groups 4 and 5.

In contrast, the effect of Genotype Groups 1 and 2 on increasing anthropometric and biochemical markers consistently were lower than the other groups (3, 4, and 5).

Discussion

The present study focused on the risk of CMDs through various polymorphisms, metabolic, and oxidative status parameters. To reach this aim, we utilized the GSEM analysis. We observed that the baseline level of anthropometric and clinical parameters in the CMD groups was significantly higher at the recruitment phase. The pathogenesis of CMDs is driven by a complex metabolic dysregulation , resulting in fundamental disturbances of fat deposition, insulin resistance, and increased arterial stiffness and vascular constriction, which lead to higher mortality rates in developed societies and developing economies[4].

A sedentary lifestyle and unhealthy dietary patterns may trigger metabolic dysregulation[4]. In our study, carriers of the recessive NQO1 genotype exhibited significantly lower physical activity than those with the dominant genotype, and no notable differences were observed in HEI, DII, and Med Diet indices; however, the Med Diet score was below the median score range in participants. Although, the impact of the Med Diet on cardiometabolic profiles varies, studies suggest the Med Diet may promote weight loss and lower BMI[29,30].

Our GSEM analysis showed that FBS had no significant direct effect on T2DM or other CMDs. However, obesity and OSI directly influenced T2DM, indicating that lipid dysregulation and inflammation could strongly drive T2DM development, with glucose contributing indirectly to CMDs. Investigations showed that redox imbalance and oxidative stress, particularly in aging, contribute to CMDs[4,8]. Evidence suggests obesity plays

Cox regression models		Genotype groups (HR (95%CI))				
		Group 1# (Nrf2 GT) *N* = 106(20.85%)	Group 2 (NQO1 CT) *N* = 101(19.84)	Group 3 (Nrf2 TT or NQO1 TT) *N* = 27(5.3%)	Group4 (Nrf2 GT & NQO1 CT) *N* = 84(16.5%)	Group 5 (Nrf2 GT & NQO1 TT) or (NQO1 CT & Nrf2 TT) *N* = 26(5.11%)
HTN	Crude	0.75(0.4_1.2) *P* = 0.30	1.31(0.8_1.90) *P* = 0.22	**2.50(1.4_4.3)***P* = 0.001	1.25(0.8_1.9) *P* = 0.31	1.75(0.9_3.2) *P* = 0.08
	Model1	0.78(0.4_1.2) *P* = 0.16	1.27(0.8_1.9) *P* = 0.18	**2.67(1.5_4.6)***P* = 0.008	1.26(0.8_1.9) *P* = 0.20	1.59(0.8_3.0) *P* = 0.18
	Model2	0.72(0.4_1.1) *P* = 0.11	1.20(0.7_1.8) *P* = 0.56	**2.45(1.4_4.2)***P* = 0.0009	1.13(0.7_1.8) *P* = 0.61	1.47(0.8_2.7) *P* = 0.27
	Model3	0.74(0.4_1.2) *P* = 0.20	1.21(0.8_1.8) *P* = 0.59	**2.57(1.4_4.4)***P* = 0.0007	1.19(0.7_1.9) *P* = 0.62	1.48(0.7_2.8) *P* = 0.32
	Model4	0.80(0.5_1.4) *P* = 0.21	1.36(0.8_2.2) *P* = 0.39	**3.01(1.5_5.5)***P* = 0.0003	1.33(0.7_2.3) *P* = 0.36	1.65(0.8_3.3) *P* = 0.20
	Model5	0.72(0.4_1.2) *P* = 0.22	1.32(0.8_2.2) *P* = 0.42	**2.82(1.4_5.3)***P* = 0.0006	1.16(0.6_2.0) *P* = 0.55	1.43(0.7_2.9) *P* = 0.28
T2DM	Crude	**2.14(1.3_3.5)***P* = 0.002	1.50(0.9_2.6) *P* = 0.32	0.70(0.7_2.3) *P* = 0.42	**3.34(2.1_5.4)***P* = 0.0004	**2.6(1.3_5.2)***P* = 0.02
	Model1	**2.07(1.2_3.4)***P* = 0.003	1.51(0.9_2.6) *P* = 0.28	0.62(0.2_2.1) *P* = 0.34	**3.5(2.1_5.6)***P* = 0.0001	**2.7(1.3_5.5)***P* = 0.01
	Model2	**2.04(1.2_3.4)***P* = 0.004	1.39(0.8_2.4) *P* = 0.56	0.60(0.2_2.0) *P* = 0.21	**3.02(1.8_5.0)***P* = 0.0004	**2.3(1.1_4.7)***P* = 0.04
	Model3	**1.80(1.1_3.0)***P* = 0.02	1.43(0.8_2.5) *P* = 0.63	0.63(0.1_2.0) *P* = 0.34	**2.92(1.7_5.0)***P* = 0.0009	1.92(0.9_4.1) *P* = 0.29
	Model4	**1.75(1.0_3.2)***P* = 0.03	1.44(0.8_2.6) *P* = 0.53	0.46(0.1_2.0) *P* = 0.22	**2.73(1.5_5.0)***P* = 0.001	1.76(0.8_3.9) *P* = 0.37
	Model5	**1.97(1.2_3.6)***P* = 0.01	1.63(0.8_3.1) *P* = 0.22	0.55(0.1_2.5) *P* = 0.10	**3.15(1.7_5.9)***P* = 0.0005	1.99(0.9_4.5) *P* = 0.51
CVD	Crude	1.36(0.9_2.1) *P* = 0.26	0.75(0.4_1.3) *P* = 0.52	0.79(0.3_2.0) *P* = 0.74	1.12(0.7_1.8) *P* = 0.73	1.30(0.6_2.7) *P* = 0.54
	Model1	1.35(0.9_2.1) *P* = 0.25	0.72(0.4_1.2) *P* = 0.36	0.92(0.4_2.3) *P* = 0.84	1.16(0.7_1.9) *P* = 0.64	1.16(0.5_2.5) *P* = 0.70
	Model2	1.35(0.8_2.1) *P* = 0.21	0.65(0.3_1.2) *P* = 0.15	0.89(0.3_2.2) *P* = 0.75	1.18(0.6_2.0) *P* = 0.54	1.24(0.5_2.7) *P* = 0.66
	Model3	1.00(0.6_1.6) *P* = 1.00	**0.55(0.3_0.9)***P* = 0.04	0.7(0.2_1.8) *P* = 0.67	0.80(0.4_1.4) *P* = 0.30	1.0(0.5_2.2) *P* = 1.00
	Model4	1.06(0.6_1.8) *P* = 0.78	0.6(0.6_1.1) *P* = 0.86	0.81(0.3_2.2) *P* = 0.81	0.86(0.4_1.6) *P* = 0.55	1.11(0.5_2.5) *P* = 0.77
	Model5	0.96(0.9_1.5) *P* = 0.91	0.57(0.3_1.0) *P* = 0.87	0.75(0.2_2.0) *P* = 0.63	0.70(0.1_1.2) *P* = 0.18	0.94(0.4_2.1) *P* = 0.95

Table 3. Association between Nrf2 and NQO1 genotype groups and the risk of CMDs: Results from unadjusted and multivariable-adjusted cox models. #The common homozygous genotypes, (GG) of Nrf2 + (CC) of NQO1, as genotype Group 0 (165 (32.4%), were considered as the reference categories in the statistical models. Model 1 adjusted for age, gender, and physical activity (METs), DII, HEI, and Mediterranean diet score. Model 2 adjusted for Model 1 + BMI, WC, HC, and FMI. Model 3 adjusted for Model 2 + TG, TC, LDL-C, and FBS. Model 4 adjusted for Model 3 + AST, GGT, Urea, and Creatinine. Model 5 adjusted for Model 4 + Uric Acid, CRP, MDA, TOS, OSI, and TAC. Significant values are in bold. The significant results are bolded (P-value 0.05)

Determinants		Cardiometabolic diseases		
		HTN (N=123)	T2DM(N=119)	CVD(N=118)
		OR(95% CI)	OR(95% CI)	OR(95% CI)
Nrf2	*GT*	0.78 (0.53 _ 1.15) P=0.20	**2.82(1.59 – 4.13) P=0.0004**	0.81(0.52 – 1.21) P=0.41
	TT	**3.24(1.37 – 7.60) P=0.007**	1.28(0.41 – 3.15) P=0.33	1.11(0.39 – 2.87) P=0.77
NQO1	*CT*	**1.46 (1.01 _ 2.14) P=0.04**	**1.97 (1.28 _ 3.01) P=0.001**	0.82 (0.55 _ 1.23) P=0.26
	TT	1.77 (0.82 _3.82) P=0.14	1.32 (0.55 _ 3.22) P=0.47	0.82 (0.35 _ 1.97) P=0.30
Obesity		1.49 (0.53 – 1.15) P=0.19	**1.75 (1.36 _2.27) P=0.0009**	0.92 (0.74 _1.21) P=0.43
FBS		**1.005 (1.00 _1.01) P=0.04**	**1.01 (1.01_ 1.02) P=0.01**	**1.006 (1.00 _ 1.01) P=0.008**
OSI		**2.30 (1.75 _ 3.04) P<0.001**	**2.36 (1.75 _3.19) P<0.001**	**3.07 (2.12 _ 4.39) P<0.001**
Dyslipidemia		0.99 (0.99 _ 1.002) P=0.11	1.006 (0.99 _1.01) P=0.69	**1.01(1.004 _ 1.02) P=0.04**
FLI		**1.07 (1.01 _ 1.14) P=0.03**	**1.29 (1.17 _1.43) P<0.001**	1.002 (0.96 _ 1.05) P=0.22
LFT		0.99 (0.980 _ 1.01) P=0.19	**1.041 (1.020_ 1.062) P=0.03**	0.97 (0.95 _ 0.99) P=0.16
Uric acid		1.05 (0.94_ 1.14) P=0.32	0.99 (0.88 _1.09) P=0.83	**1.17 (1.04 _ 1.30) P=0.007**
KFT		0.95 (0.86 _ 1.03) P=0.26	0.95 (0.86_ 1.03) P=0.24	0.95 (0.86 _ 1.03) P=0.20

Table 4. GSEM analysis of direct effects of Nrf2 and NQO1 genotypes and cardiometabolic determinants (latent and observed variables) on CMD outcomes. The common homozygous genotypes (GG) of Nrf2 and (CC) of NQO1 were considered as the reference categories in the statistical models. FBS: fasting blood sugar, OSI oxidative stress index, FLI fatty liver index, LFT liver function test, KFT Kidney function test. Significant values are in bold. The significant results are bolded (P-value 0.05)

Path to the cardiometabolic disease		Nrf2				NQO1			
		GT		TT		CT		TT	
		Indirect effect OR(95%CI)	Total effect OR(95%CI)	Indirect effect OR(95%CI)	Total effect OR(95%CI)	Indirect effect OR(95%CI)	Total effect OR(95%CI)	Indirect effect OR(95%CI)	Total effect OR(95%CI)
SNP -> obesity -> disease	HTN	1.02(0.99_1.11) P = 0.49	0.79(0.53_1.17) P = 0.24	1.09(0.93_1.14) P = 0.09	**3.35(1.39_7.83)** P = 0.006	1.02(0.99_1.07) P = 0.31	**1.46(1.01_2.14)** P = 0.04	1.08(0.96_1.23) P = 0.22	1.77(0.82_3.82) P = 014
	T2DM	1.08(0.99_1.20) P = 0.11	**2.98(1.71_4.48)** P = 0.04	**1.43(1.12_1.90)** P = 0.007	1.33(0.41_3.27) P = 0.59	**1.12(1.01_1.23)** P = 0.02	**1.97(1.28_3.01)** P = 0.001	**1.39(1.09_1.77)** P = 0.007	1.32(0.55_3.22) P = 0.53
	CVD	0.99(0.95_1.49) P = 0.93	0.82(0.55_1.23) P = 0.33	0.92(0.78_1.08) P = 0.31	1.11(0.43_2.90) P = 0.83	0.99(0.94_1.49) P = 0.93	0.82(0.55_1.23) P = 0.33	0.95(0.88_1.09) P = 0.34	0.82(0.35_1.97) P = 0.65
SNP -> FBS -> disease	HTN	1.03(0.99_1.08) P = 0.18	0.80(0.54_1.16) P = 0.25	1.004(0.95_1.11 P = 0.91	**3.28(1.39_7.60)** P = 0.005	1.01(0.99_1.04) P = 0.42	**1.49(1.01_2.16)** P = 0.03	1.04(0.95_1.06) P = 0.16	1.77(0.83_3.82) P = 0.14
	T2DM	1.05(0.95_1.16) P = 0.33	**2.98(1.29_3.17)** P = 0.04	1.01(0.83_1.25) P = 0.92	1.33(0.55_3.24) P = 0.52	1.05(0.95_1.16) P = 0.33	**2.00(1.30_3.10)** P = 0.001	1.01(0.83_1.25) P = 0.92	1.34(0.55_3.25) P = 0.51
	CVD	1.01(0.98_1.05) P = 0.57	0.81(0.53_1.22) P = 0.32	1.03(0.94_2.05) P = 0.88	1.11(0.41_2.90) P = 0.83	1.01(0.98_1.05) P = 0.57	0.80(0.53_1.22) P = 0.29	1.004(0.94_2.01P = 0.98	0.83(0.37_1.99) P = 0.66
SNP -> OSI -> disease	HTN	**1.61(1.31_2.03)** P < 0.001	0.82(0.53_1.12) P = 0.29	**2.92(1.80_4.71)** P = 0.03	**5.11(1.94_13.4)** P = 0.0009	**1.26(1.07_1.48)** P = 0.005	**1.54(1.01_1.57)** P = 0.0001	**1.82(1.23_2.46)** P = 0.0007	**2.25(1.01_3.78)** P = 0.01
	T2DM	**1.51(1.07_1.66)** P = 0.0002	**4.94(1.29_6.16)** P = 0.03	1.79(0.84_2.57) P = 0.07	1.48(0.54_3.94) P = 0.43	**1.79(1.23_2.56)** P = 0.001	**2.03(1.29_3.19)** P = 0.002	**1.36(1.09_1.68)** P = 0.005	1.51(0.54_4.10) P = 0.42
	CVD	**1.36(1.09_1.69)** P = 0.006	0.91(0.52_1.37) P = 0.70	**3.39(1.91_5.94)** P = 0.04	1.35(0.48_3.86) P = 0.57	**1.36(1.09_1.68)** P = 0.005	0.91(0.52_1.36) P = 0.70	**2.14(1.34_3.40)** P = 0.001	0.97(0.33_2.95) P = 0.95
SNP -> dyslipidemia -> disease	HTN	0.98(0.90_1.06) P = 0.62	0.79(0.53_1.17) P = 0.24	0.99(0.93_1.04) P = 0.72	**3.22(1.37_7.50**) P = 0.007	0.94(0.85_1.03) P = 0.20	**1.46(1.02_2.20)** P = 0.04	0.95(0.86_1.04) P = 0.29	1.75(0.83_3.79) P = 0.14
	T2DM	1.05(0.96_1.16) P = 0.31	**2.81(1.59_3.91)** P = 0.03	1.04(0.96_1.11) P = 0.28	1.33(0.54_3.24) P = 0.53	1.07(0.98_1.13) P = 0.06	**1.93(1.28_2.90)** P = 0.001	1.06(0.97_1.18) P = 0.24	1.31(0.55_3.12) P = 0.54
	CVD	**1.13(1.01_1.25)** P = 0.02	0.82(0.54_1.23) P = 0.34	1.08(0.96_1.22) P = 0.20	1.12(0.41_2.88) P = 0.81	**1.17(1.04_1.32)** P = 0.009	0.83(0.54_1.25) P = 0.38	**1.16(1.00_1.34)** P = 0.04	0.83(0.33_2.01) P = 0.68
SNP -> FLI -> disease	HTN	1.04(0.98_1.10) P = 0.18	0.78(0.53_1.17) P = 0.021	**1.11(1.00_1.25)** P = 0.04	**3.33(1.33_7.42)** P = 0.006	1.07(0.99_1.16) P = 0.09	**1.54(1.06_2.32)** P = 0.03	1.04(0.93_1.17) P = 0.50	**1.79(1.21_3.87)** P = 0.04
	T2DM	**1.14(1.04_1.27)** P = 0.01	**3.12(1.83_3.93)** P = 0.04	1.17(0.80_1.71) P = 0.41	1.32(0.55_3.21) P = 0.52	**1.28(1.04_1.60)** P = 0.02	**2.29(1.42_3.65)** P = 0.0005	1.17(0.79_1.74) P = 0.43	1.43(0.55_3.81) P = 0.46
	CVD	0.99(0.98_1.02) P = 0.32	0.69(0.52_1.21) P = 0.08	0.99(0.94_1.05) P = 0.72	1.11(0.41_2.86) P = 0.83	1.002(0.96_1.05 P = 0.93	0.81(0.53_1.23) P = 0.32	1.001(0.98_1.03 P = 0.93	0.83(0.34_1.99) P = 0.67
SNP -> LFT -> disease	HTN	1.01(0.94_1.06) P = 0.74	0.79(0.53_1.17) P = 0.24	1.005(0.88_1.06 P = 0.91	**3.29(1.38_7.52)** P = 0.005	0.99(0.91_1.05) P = 0.78	**1.46(1.00_2.18)** P = 0.04	0.98(0.88_1.08) P = 0.69	1.75(0.83_3.79) P = 0.12
	T2DM	**1.14(1.03_1.27)** P = 0.01	**2.92(1.93_4.00)** P = 0.03	1.12(0.83_1.31) P = 0.33	1.27(0.54_3.15) P = 0.59	**1.17(1.05_1.31)** P = 0.005	**1.97(1.28_3.01)** P = 0.002	**1.26(1.02_1.46)** P = 0.01	1.28(0.52_3.15) P = 0.59
	CVD	**0.89(0.80_0.99)** P = 0.03	0.80(0.53_1.21) P = 0.28	0.90(0.73_1.07) P = 0.28	1.11(0.42_2.87) P = 0.83	**0.89(0.79_0.96)** P = 0.02	0.82(0.53_1.23) P = 0.36	0.85(0.70_1.01) P = 0.08	0.81(0.33_1.98) P = 0.63
SNP -> Uric acid -> disease	HTN	1.06(0.94_1.12) P = 0.19	0.79(0.53_1.17) P = 0.24	1.03(0.95_1.19) P = 0.60	**3.20(1.36_7.42)** P = 0.007	1.02(0.95_1.11) P = 0.61	**1.46(1.00_2.18)** P = 0.04	1.03(0.93_1.16) P = 0.60	1.75(0.83_3.79) P = 0.14
	T2DM	0.97(0.89_1.06) P = 0.49	**2.83(1.72_3.92)** P = 0.04	0.97(0.87_1.07) P = 0.56	1.26(0.55_3.10) P = 0.60	0.99(0.91_1.06) P = 0.79	**1.92(1.27_2.91)** P = 0.003	0.98(0.87_1.11) P = 0.74	1.27(0.52_3.09) P = 0.59
	CVD	**1.09(1.01_1.21)** P = 0.04	0.81(0.53_1.21) P = 0.31	1.04(0.96_1.15) P = 0.39	1.11(0.41_2.86) P = 0.82	**1.13(1.01_1.22)** P = 0.01	0.82(0.53_1.25) P = 0.35	1.07(0.96_1.19) P = 0.21	0.82(0.33_1.98) P = 0.66
SNP -> KFT -> disease	HTN	0.96(0.87_1.06) P = 0.41	0.79(0.53_1.17) P = 0.24	0.94(0.84_1.05) P = 0.27	**3.21(1.36_7.45**) P = 0.006	0.95(0.86_1.03) P = 0.48	**1.49(1.02_2.18)** P = 0.03	0.94(0.84_1.04) P = 0.25	1.77(0.83_3.79) P = 0.13
	T2DM	0.91(0.83_1.00) P = 0.05	**2.85(1.82_3.92)** P = 0.04	0.92(0.78_1.02) P = 0.22	1.30(0.55_3.14) P = 0.56	0.91(0.83_1.01) P = 0.06	**1.94(1.28_2.93)** P = 0.001	0.92(0.78_1.02) P = 0.22	1.31(0.54_3.13) P = 0.54
	CVD	1.005(0.90_1.1) P = 0.92	0.80(0.53_1.21) P = 0.28	1.006(0.86_1.16 P = 0.93	1.11(0.42_2.86) P = 0.83	1.03(0.95_1.12) P = 0.26	0.82(0.53_1.23) P = 0.38	1.04(0.93_1.16) P = 0.48	0.83(0.34_1.98) P = 0.67

Table 5. GSEM analysis of indirect and total of Nrf2 and NQO1 genotypes and cardiometabolic determinants (latent and observed variables) on CMD outcomes. FBS fasting blood sugar, OSI oxidative stress index, FLI fatty liver index, LFT liver function test, KFT Kidney function test. Significant values are in bold. The significant results are bolded (P-value 0.05)

a central role[31,32]. Free fatty acid accumulation in adipocytes triggers chronic inflammation in white adipose tissue[32], leading to insulin resistance and diabetes[33].

In obesity-related chronic inflammation, adipocytes secrete proinflammatory cytokines (IL-1β, IL-6, TNF-α), exacerbating inflammation and elevating ROS[34,35]. Excess ROS activates phosphatidylinositol-3-kinase (PI3K), phosphorylating protein kinase B (AKT), which disrupts the Keap1-Nrf2 complex, freeing Nrf2. Nrf2 translocates to the nucleus, binds the antioxidant response element, and induces antioxidant gene expression[36].

Nrf2 prevents apoptosis and inflammation by downregulating NF-κB and promotes detoxification and redox balance via increased SOD-1, CAT, HO-1, and NQO1[33].

NQO1, a cytoprotective enzyme, catalyzes the two-electron reduction of quinones and related redox-active compounds. This process prevents their involvement in redox cycling, thereby reducing ROS generation.

By mitigating oxidative stress, NQO1 contributes to protecting against chronic diseases such as cancer, neurodegenerative disorders, and metabolic syndromes[37].

In our study, T allele carriers (heterozygotes and homozygotes) of Nrf2 and NQO1 showed significantly higher FBS, BMI, WC, and HC than the dominant genotypes. Genotype Group 4 had the highest T2DM risk. GSEM analysis revealed that Nrf2 GT increases T2DM risk through obesity, FBS, IOS, FLI, and LFT pathways, while T2DM risk is indirectly affected by obesity, IOS, and LFT in the Nrf2 and NQO1 TT genotype group.

Unlike our prior study among Iraq's Kurdish population, which found the Nrf2 T allele reduced obesity and T2DM risk in normal-BMI individuals[13], current results show increased T2DM risk in T allele carriers. Tao et al.[17] reported no significant difference in the frequency of Nrf2 rs6721961 in Chinese Han metabolic syndrome patients. Various genetic backgrounds, gene-environment interaction, nutritional and physical activity styles may drive this difference.

Conversely, in line with the present study, Wang, Jia, and their colleagues in separate investigations found that the Nrf2 rs6721961 polymorphism contributed to T2DM and cardiovascular complications[14,15]. Experimental models under a hyperglycemic situation depicted the β-cells by the formation of three disulfide bonds per one insulin molecule, vigorously release ROS. The cells to defend against this issue, early-stage T2DM rat cells increased Nrf2 levels, which supports the protective Nrf2 role[38].

Reports on NQO1 (rs1800566) are also contradictory. Jiménez-Osorio's case-control study found no association between NQO1 variants with T2DM or obesity in Mexicans, without evaluating dietary or lifestyle factors[39]. Our results showed that NQO1 T allele carriers (heterozygotes and homozygotes), particularly in genotype Groups 3, 4, and 5 (along with Nrf2), positively influenced inflammatory, oxidative stress, lipid profile, FBS, and body adiposity markers.

Several studies suggested that the NQO1*2 null polymorphism (rs1800566) is linked to increased risks of adverse lipid profiles, coronary artery disease, and elevated glucose levels in metabolic syndrome[40,41]. For instance, Gaikwad et al. showed that NQO1-null mice developed insulin resistance, leading to a T2DM-like phenotype[42].

Consistent with Kunnas et al.'s study, which linked the Nrf2 rs6721961 T allele to HTN without assessing metabolic profiles[43], our Cox regression analysis showed that genotype Group 3 had a significant association with HTN risk in both crude and adjusted models. GSEM analysis revealed that the TT genotype of Nrf2 and NQO1 had a significant direct and indirect effect on increasing HTN risk via obesity, FBS, FLI, and uric acid pathways; however, the direct effect was stronger in Nrf2 TT genotype carriers.

In obesity, the renin-angiotensin-aldosterone system (RAAS) promotes inflammation and vascular dysfunction, contributing to high blood pressure. RAAS dysfunction has been observed in hypertensive rat models with downregulated Nrf2 and its antioxidant enzymes like NQO1[44,45]. Nrf2, by upregulating NQO1, inhibits the increase of NOX1, eNO, and NO, thus reducing oxidative stress and regulating blood pressure[33].

As noted, FLI mediates the impact of Nrf2 and NQO1 polymorphisms on increased CMDs risk in this study. Wakabayashi et al. (2023) investigated Nrf2 and NQO1 induction in adipose tissue and liver cells to regulate oxidative status and lipid accumulation in hepatocytes. Their results showed that enhancing Nrf2 expression in hepatocytes (but not adipose tissue) reduced liver fat, improved insulin resistance, lowered ALT (a marker of liver damage), and decreased liver weight. Additionally, increased NQO1 expression stabilized Nrf2 against ubiquitination and reduced oxidative stress[46].

Therefore, it might if there is no direct association between Nrf2 and NQO1 association with CMDs, the liver dysfunction in the presence of polymorphisms would cause multiple metabolic disorders.

Atherosclerosis (AS) is a chronic inflammatory disease underlying cardiovascular disorders. Nrf2 plays a dual, context-dependent role in AS development. It protects against AS by activating antioxidant pathways, suppressing LDL oxidation, reducing ROS levels, and preventing foam cell formation through genes like peroxiredoxin 1 (Prdx1). Loss of Nrf2 worsens oxidative stress, inflammation, and atherosclerotic lesions, as seen in LDLR−/− and Nrf2−/− mouse models[47,48].

However, under certain conditions, Nrf2 may promote AS progression. For instance, Nrf2 deletion in ApoE−/− mice reduces plaque burden, likely by decreasing CD36 expression and ox-LDL uptake. Cholesterol crystals can activate Nrf2, enhancing inflammasome signaling and IL-1β-mediated vascular inflammation, thus exacerbating AS[49,50].

In this study, no direct association was found between Nrf2 and NQO1 polymorphisms and CVD. However, CVD risk was indirectly increased by IOS, uric acid, and dyslipidemia in individuals with the Nrf2 GT and NQO1 CT and TT genotypes.

In 2022, Yu et al. demonstrated in ApoE−/− mice, high uric acid levels inhibit the Nrf2/SLC7A11/GPx4 pathway, increasing ferroptosis and promoting atherosclerotic plaque formation. Additionally, high uric acid decreases GSH levels and elevates lipid ROS in macrophage-derived foam cells[16].

Finally, the role of aging in the development of CMDs should not be ignored. NQO1 levels decline with age, reducing defense against oxidative stress. Researchers are exploring strategies to enhance NQO1 expression in the liver and brain to protect against oxidative damage[37].

Strengths and limitations

This study is the first to investigate the association of Nrf2 and NQO1 polymorphisms with CMDs risk factors, dietary patterns, and clinical parameters in the Kurdish Iranian population using a cohort design with an 8-year follow-up. The combination of multiple risk factors, including genotypes, biochemical, anthropometric, and nutritional parameters, represents a key strength for a more comprehensive examination of gene-environment interactions in metabolic disorders.

However, several limitations must be noted. The sample size was sufficient for the main analyses, but it may limit statistical power for detecting subgroup-specific associations. This study focused on selected SNPs

without assessing functional biomarkers such as gene expression or enzyme activity, and specific inflammation biomarkers, which could provide further insight into the biological relevance of the observed associations.

Future studies with larger and more diverse populations and functional investigations at the transcriptomic and proteomic levels are needed to confirm these results and enhance understanding of the molecular pathways involved in CMDs.

Conclusion

This study suggests that genetic variants of Nrf2 (rs6721961) and NQO1 (rs1800566) are significantly associated with the risk of developing CMDs, particularly T2DM and HTN. Individuals with recessive genotypes (T allele carriers) of Nrf2 and NQO1 displayed an impaired metabolic profile (FBS, lipid, LFT, and KFT), increased anthropometric measures, and oxidative stress biomarkers, highlighting their role in metabolic and redox homeostasis.

Survival analysis showed that the Nrf2 and NQO1 TT genotype group had the highest risk for HTN incidence, while genotype Groups 1 and 4 contributed to an increased risk of T2DM.

GSEM analysis revealed that OSI directly contributed to CMDs development, more than obesity or FLI. The indirect effects of Nrf2 polymorphisms, mediated by obesity, OSI, and FLI, increased HTN and T2DM risk. Similarly, the NQO1 TT genotype did not show a direct association with disease incidence, but had significant indirect effects on HTN via oxidative stress and liver dysfunction.

Although no direct link was found between the polymorphisms and CVD, indirect pathways involving OSI, uric acid, and dyslipidemia significantly contributed to CVD risk. Glucose levels did not show a strong direct association with CMDs but may affect it indirectly through obesity-related inflammation and oxidative stress. In addition, a lower grade of the Mediterranean diet may exacerbate metabolic dysregulation.

Overall, our findings highlight how Nrf2 and NQO1 genetic variations influence susceptibility to CMDs through interactions with oxidative stress, inflammation, and metabolic dysfunction, offering insights for early risk stratification and personalized preventive strategies.

Data availability

The datasets analyzed during the current study are not publicly available due privacy concerns but are available from the corresponding author on reasonable request.

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Author contributions

MK: collected the data and samples. Did laboratory tests, analyzed the data, and wrote the first draft of the manuscript. MA collected the data and samples and did laboratory analysis. M MN: Analyzed the data. KH Y, DS, and E SH: revised the manuscript. ZR: Designed the study and revised the final version of the manuscript. All authors have read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

We confirm that all methods relevant to human participants were performed in accordance with the Declaration of Helsinki and approved by the research ethics committee of Kermanshah University of Medical Sciences (Ethics code: IR.KUMS.MED.REC.1402.328). The study protocol was fully explained to all

participants, and each individual provided written informed consent to participate in the research.

Competing interests
The authors declare no competing interests.

Additional information
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