COX-2 rs20417 polymorphism and susceptibility to type 2 diabetes mellitus

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Abstract. Type 2 diabetes mellitus (T2DM) is a multifactorial disease influenced by environmental and genetic factors. The present case-control study aimed to evaluate the association between three cyclooxygenase-2 (*COX-2*) gene polymorphisms (rs689466, rs20417 and rs2066826) and the susceptibility to T2DM in the Jordanian population. A total of 202 participants were enrolled, comprising 101 patients with T2DM and 101 healthy controls matched by sex and age (±5 years). Genotyping was performed using PCR-restriction fragment length polymorphism, and clinical and biochemical parameters were assessed. The rs20417 polymorphism showed a significant association with T2DM. In multivariate logistic regression analysis adjusted for age, sex, body mass index, hypertension and triglyceride levels, individuals carrying the CG genotype were more likely to develop T2DM [odds ratio (OR)=5.09; 95% CI: 1.18-21.96; P=0.0290], as did those with the CC genotype (OR=5.39; 95% CI: 1.34-21.72; P=0.0178), compared with those with the GG genotype. No significant associations were observed for rs689466 or rs2066826. Haplotype analysis identified the T-C-C haplotype (rs689466-rs20417-rs2066826) as a risk haplotype associated with increased susceptibility to T2DM (OR=1.52; 95% CI: 1.01-2.30; P=0.046). These findings suggest that the *COX-2* rs20417 polymorphism is independently associated with T2DM risk in Jordanians, supporting a role for COX-2-mediated inflammatory pathways in the pathogenesis of diabetes.

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Abbreviations: BMI, body mass index; COX, cyclooxygenase; RFLP, restriction fragment length polymorphism; SNP, single-nucleotide polymorphism; T2DM, type 2 diabetes mellitus

Key words: T2DM, COX-2, rs689466, rs20417, rs2066826, SNP

Introduction

Diabetes mellitus (DM) is a sustained metabolic disorder with a rising global incidence, posing a significant public health challenge worldwide (1,2). DM is generally divided into three main types: i) Type 1 DM, formerly known as juvenile or insulin-dependent diabetes, results from the body's inability to produce insulin; ii) type 2 DM (T2DM) begins with insulin resistance, may progress to decreased insulin production and is mostly related to obesity and physical inactivity; and iii) gestational diabetes arises in pregnant women who lack a history of diabetes, resulting in elevated blood glucose levels (3). The most prevalent form of the disease is T2DM, which is primarily associated with insulin resistance and lifestyle habits, including sedentary behavior, poor dietary choices and irregular sleep patterns (4). In Jordan, the prevalence of T2DM increased by 14.3% from 1990 to 2020 and is projected to rise by a further 28.8% between 2020 and 2050 (5).

T2DM is commonly associated with low-grade systemic inflammation, characterized by increased serum cytokine levels, which is believed to be pivotal in its pathogenesis (6). Low-grade inflammation mediated by cyclooxygenase (COX) pathways, along with oxidative stress, is increasingly recognized as an important factor influencing the development and progression of T2DM (7). COX has two isoforms: COX-2 functions as the inducible variant, while COX-1 is expressed under normal physiological conditions. The COX enzymes facilitate the biosynthesis of prostaglandins using arachidonic acid as a substrate (8). COX-1 sustains physiological activities, including gastric protection and platelet aggregation, whereas COX-2 is upregulated during inflammation and enhances the synthesis of pro-inflammatory prostaglandins (9,10). Inflammation associated with diabetes has been linked to IL-1β (11), which stimulates the secretion of prostaglandin E2 (PGE2) via increasing *COX-2* gene expression (also known as prostaglandin endoperoxide synthase 2) (12). Higher PGE2 levels have been shown to compromise pancreatic β-cell function, hence lowering insulin secretion levels and increasing susceptibility to the development of diabetes (13).

T2DM arises from the intricate interaction of metabolic, environmental and genetic risk factors. Individuals with a strong familial predisposition to diabetes, older age, obesity

and sedentary lifestyles are at the highest risk for developing the disease (14). At the genetic level, single-nucleotide polymorphisms (SNPs) in multiple genes have been associated with increased vulnerability to T2DM (15-17). One of these genes is *COX-2* (18,19).

The *COX-2* gene includes 10 exons separated by 9 introns, a 3'-untranslated region (3'-UTR) and a 5'-flanking region (20). Polymorphisms in the *COX-2* gene can affect enzyme expression and activity, thereby modifying the risk of developing cancer and other inflammatory-related diseases (21).

Two *COX-2* gene variations, rs20417 in the promoter region and rs2066826 in intron 6, were found to have a notable association with T2DM in Pima Indians (19). Furthermore, a study conducted in Turkey found that the rs5275 variant of the *COX-2* gene, located in the 3'-UTR, may contribute to the etiology or influence the risk of developing T2DM (18). By contrast, the rs689466 variant of the same gene showed no significant association with T2DM risk in either Pima Indians or the Turkish population (18,19). Building on these findings, the present study aimed to investigate the association of three key SNPs (rs689466, rs20417 and rs2066826) with the development of T2DM in the Jordanian population. Although the rs689466 variant showed no association with T2DM in previous studies, it was included in the present study to verify these findings in the Jordanian population and to assess its contribution to haplotype analysis.

Materials and methods

Study design and patient recruitment. The present case-control study included 202 participants from King Abdullah University Hospital (teaching hospital affiliated with Jordan University of Science and Technology; Irbid, Jordan) between September 2023 and February 2024. All participants were aged ≥25 years with confirmed T2DM diagnosis [hemoglobin A1c (HbA1c) ≥6.5%] for patients, and HbA1c <5.7% for healthy individuals who served as controls. Exclusion criteria included individuals <25 years old, pregnant women and individuals with HbA1c values between 5.7 and 6.5% (prediabetic range) to ensure clear metabolic distinction between cases and controls. Demographic data (age, sex and smoking status) were recorded, and clinical results (such as kidney and liver function tests and HbA1c) were collected from electronic medical records. Participants were divided into two groups: 101 controls and 101 individuals with previously diagnosed T2DM. Controls were matched to patients with T2DM by sex and age (±5 years). Body mass index (BMI) was not used as a matching variable, but it was adjusted for in the multivariate analyses.

Blood sample collection. A venous blood sample (3-4 ml) was obtained from each participant using sterile tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant to prevent clotting. Immediately after collection, each sample was gently inverted several times to ensure thorough mixing with EDTA. The samples were then stored at 4˚C to maintain the integrity and stability of genomic DNA until further processing and extraction.

DNA extraction. Genomic DNA was extracted from whole blood samples using the QIAamp DNA Blood Mini Kit (cat. no. 51104; Qiagen GmbH), following the manufacturer's protocol. Upon completion of the extraction process, DNA concentration and purity were measured using the ND-2000 NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc.). To ensure long-term preservation and maintain the integrity of the extracted DNA, all samples were stored at -20˚C for further analysis.

Genotyping. Three SNPs within the *COX-2* gene (rs689466, rs20417 and rs2066826) were identified using the PCR-restriction fragment length polymorphism (PCR-RFLP) method.

Each SNP region was amplified by conventional PCR (Bioer GeneExplorer™ Thermal Cycler; Hangzhou Bioer Technology Co., Ltd.) using specifically designed primers (Table I). Reactions were prepared in a total volume of 25 μl using 2X GoTaq® Green Master Mix (Promega Corporation). The reactions contained 1 μl of each primer pair (1 μM), 2 μl (10 ng) of DNA template, 8.5 μl of nuclease-free water and 12.5 μl of 2X PCR Master Mix solution. The PCR conditions were optimized for each primer set to ensure high specificity and efficient amplification of the genomic regions flanking the target SNPs. Thermal cycling included an initial denaturation at 95˚C for 3 min, followed by 34 cycles of 95˚C for 30 sec, 60˚C for 30 sec and 72˚C for 30 sec, with a final extension at 72˚C for 5 min. PCR amplification was verified by running the products in a 2% agarose gel stained with 5 μl RedSafe™ Nucleic Acid Staining Solution (20,000X; iNtRON Biotechnology, Inc.).

The PCR-amplified products for each SNP were subjected to specific restriction enzyme digestion targeting the polymorphic sites as listed in Table I. The reactions were incubated at 37˚C for 6 h following the enzyme-specific protocol. After restriction enzyme digestion, the PCR products were resolved on 3% agarose gels stained with RedSafe Nucleic Acid Staining Solution and visualized under ultraviolet illumination. A DNA ladder was used to verify fragment sizes. Genotypes were determined based on the resulting fragment patterns visualized on agarose gels (Fig. S1).

To validate the PCR-RFLP results, 10 samples per SNP (30 samples total) selected to represent all observed genotypes were confirmed by Sanger sequencing (data not shown), with 100% concordance observed between the two methods.

Statistical analysis. Statistical analysis was performed using R software version 4.3.1 (Posit Software, PBC). Categorical variables are presented as n (%) and group comparisons were performed using the χ^2 test or Fisher's exact test, depending on the contingency table size and cell count. Specifically, Fisher's exact test was applied for 2x2 contingency tables or when ≥20% of the expected cell counts were <5. HbA1c levels are presented as medians [interquartile ranges (IQR)], and the comparison of their levels between control and T2DM groups was performed using the Mann-Whitney U test. Hardy-Weinberg equilibrium (HWE) was assessed for all SNPs in the control group using an exact test according to SNPstats (https://snpstats.net/start.htm). Haplotype construction was conducted using the SHEsisPlus online platform (http://shesisplus.bio-x.cn/SHEsis.html). Multivariate logistic regression analysis was performed to assess the independent association between *COX-2* polymorphisms and the risk of T2DM, while adjusting for potential

Table I. Cyclooxygenase-2 variants genotyped using PCR-RFLP.

SNP ID	Region base change	Primer sequences (5'-3')	Enzyme and recognition site	PCR product size (bp)	RFLP product (bp)
rs689466	Promoter T>C	F: AATTTGCAGGGACGCTAAA R: CCTGAGCACTACCCATGATAGA	AluI: 5'-AG/CT-3'; 3'-TC/GA-5'	352	CC:300/52; TT:352; TC:352/300/52
rs20417	Promoter G>C	F: GACGACGCTTAATAGGCTGTA R: CCATCAGAAGGCAGGAAACT	AciI: 5'-C/CGC-3'; 3'-GGC/G-5'	307	GG:307; CC:200/107; CG:307/200/107
rs2066826	Intron C>T	F: CTTTGACTGTGGGAGGATACAT R: CAGTTTGTAGCTTTGGTGGATAAA	AciI: 5'-C/CGC-3'; 3'-GGC/G-5'	427	TT: 427; CC: 309/118; CT:427/309/118

SNP, single-nucleotide polymorphism; RFLP, restriction fragment length polymorphism; F, forward; R, reverse.

confounding variables such as age, sex, BMI, hypertension and triglyceride levels. Adjusted odds ratios (ORs) and 95% CIs were calculated to estimate the strength of the associations. Given the hypothesis-driven selection of candidate SNPs based on the literature, formal correction for multiple testing (three SNPs tested for genotype and allele associations plus haplotype analysis) was not applied. This approach is consistent with recommendations for candidate gene studies with a limited number of pre-specified variants (22). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Enrolled patient characteristics. In the present study, a total of 202 participants were enrolled from the Endocrinology Clinic at King Abdullah University Hospital, including 101 patients with T2DM and 101 healthy individuals whose HbA1c level was <5.7%, serving as controls. The clinical and demographic characteristics of the study population are outlined in Table II.

Serum HbA1c levels were used as a reference to assess glycemic status in the study population. HbA1c levels were markedly elevated in the T2DM group compared with those in controls ($P<0.0001$). The median HbA1c level was 5.4% (IQR: 5.2-5.5%) in the control group and 7.2% (IQR: 6.2-8.7%) in the T2DM group, indicating a clear metabolic distinction associated with T2DM (Fig. 1).

A deliberate matching process was carried out to ensure comparability between the T2DM and healthy control groups in terms of age (±5 years) and sex. As a result, no significant differences were found between the two groups in these variables. Women comprised 60.40% of each group, while men accounted for 39.60%. The mean age was 52.75±8.12 years in the T2DM group and 50.32±12.41 years in the control group, with ~80% of participants in each group being <60 years old.

Smoking status was also assessed and did not show any significant difference between the T2DM and control groups. Among individuals with T2DM, 22.47% were current smokers, 74.16% were non-smokers and 3.37% were ex-smokers. In the control group, 18.07% were current smokers, 80.72% were non-smokers and 1.20% were former smokers.

Furthermore, BMI analysis revealed no significant difference between the two groups, indicating a comparable distribution of this key metabolic risk factor. However, a significant disparity in hypertension prevalence was observed ($P<0.0001$), with 69.31% of individuals in the T2DM group affected, compared with 34.65% in the control group.

In addition, biochemical analyses revealed a notable difference in triglyceride levels, with a higher proportion of elevated triglycerides among patients with T2DM compared with controls (64.36% vs. 43.56%, respectively; $P=0.0046$), further underscoring the metabolic disturbances commonly associated with T2DM.

Association of COX-2 genetic variants with T2DM. Allele and genotype distributions of the *COX-2* SNPs rs689466, rs20417 and rs2066826 were determined using PCR-RFLP analysis. The genotype distributions of these three SNPs were compared between the T2DM and healthy control groups, as summarized in Table III. No statistically

Table II. Baseline characteristics of study subjects and biochemical profile.

Variable	T2DM (n=101)	Healthy controls (n=101)	P-value
Age			0.7268
<60	82 (81.19)	79 (78.22)	
≥60	19 (18.81)	22 (21.78)	
Sex			1.0000
Female	61 (60.40)	61 (60.40)	
Male	40 (39.60)	40 (39.60)	
BMI			0.8219
<25	12 (11.88)	10 (9.90)	
≥25	89 (88.12)	91 (90.10)	
Smoking status[a]			0.4736
Smoker	20 (22.47)	15 (18.07)	
Non-smoker	66 (74.16)	67 (80.72)	
Ex-smoker	3 (3.37)	1 (1.20)	
Comorbidities (Yes)			
HTN	70 (69.31)	35 (34.65)	<0.0001
CKD	5 (4.95)	4 (3.96)	1.0000
IHD	7 (6.93)	4 (3.96)	0.5373
Biochemical blood test			
Cholesterol, mmol/l			0.9499
Normal (<5.2)	73 (72.28)	75 (74.26)	
Borderline high (5.2-6.2)	16 (15.84)	15 (14.85)	
High (≥6.2)	12 (11.88)	11 (10.89)	
HDL[b], mmol/l			0.1561
Normal	11 (10.89)	10 (9.90)	
Moderate risk	51 (50.50)	64 (63.37)	
High risk	39 (38.61)	27 (26.73)	
LDL, mmol/l			0.7954
Normal (0-4.40)	94 (93.07)	92 (91.09)	
High (>4.40)	7 (6.93)	9 (8.91)	
Triglycerides, mmol/l			0.0046
Normal (0-1.7)	36 (35.64)	57 (56.44)	
High (>1.7)	65 (64.36)	44 (43.56)	
Creatinine, μmol/l			1.0000
Low (<53)	8 (7.92)	8 (7.92)	
Normal (53-97)	83 (82.18)	83 (82.18)	
High (>97)	10 (9.90)	10 (9.90)	
Urea, mmol/l			0.8656
Low (<2.14)	1 (0.99)	2 (1.98)	
Normal (2.14-7.14)	88 (87.13)	89 (88.12)	
High (>7.14)	12 (11.88)	10 (9.90)	

[a]Due to missing data on smoking status, percentages were calculated based on the available responses (T2DM, n=89; healthy controls, n=83). [b]For HDL subcategories: Men (normal >1.45, moderate risk 0.9-1.45, high risk <0.9); women (normal >1.68, moderate risk 1.15-1.68, high risk <1.15). Data are presented as n (%). T2DM, type 2 diabetes mellitus; BMI, body mass index; IHD, ischemic heart disease; HTN, hypertension; CKD, chronic kidney disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

significant differences were observed for rs2066826 and rs689466 (P>0.05). HWE was assessed for all three SNPs in the control group, since deviation from HWE in cases may reflect a true disease association. The rs689466 and rs2066826 loci conformed to HWE (P=0.24 and P=0.76 respectively). A modest deviation from HWE was observed for rs20417 in controls (P=0.03), which may reflect population structure or chance variation due to sample size.

For rs2066826, the TT genotype was the least frequent in both groups, while the CC genotype was the most common.

Table III. Genotype and allele frequency for polymorphisms rs689466, rs20417 and rs2066826.

A, rs689466

SNP	T2DM (n=101)	Healthy controls (n=101)	HWE control P-value	P-value
Genotype			0.24	1.0000
TT	83 (82.18)	83 (82.18)		
TC	16 (15.84)	16 (15.84)		
CC	2 (1.98)	2 (1.98)		
Allele			NA	1.0000
T	182 (90.10)	182 (90.10)		
C	20 (9.90)	20 (9.90)		

B, rs20417

SNP	T2DM (n=101)	Healthy controls (n=101)	HWE control P-value	P-value
Genotype			0.03	0.0248
CC	68 (67.33)	57 (56.44)		
CG	30 (29.69)	31 (30.69)		
GG	3 (2.97)	13 (12.87)		
Allele			NA	0.0178
C	166 (82.18)	145 (71.78)		
G	36 (17.82)	57 (28.22)		

C, rs2066826

SNP	T2DM (n=101)	Healthy controls (n=101)	HWE control P-value	P-value
Genotype			0.76	0.2707
CC	74 (73.27)	64 (63.37)		
CT	25 (24.75)	32 (31.68)		
TT	2 (1.98)	5 (4.95)		
Allele			NA	0.1163
C	173 (85.64)	160 (79.21)		
T	29 (14.36)	42 (20.79)		

Data are presented as n (%). SNP, single-nucleotide polymorphism; T2DM, type 2 diabetes mellitus; HWE, Hardy-Weinberg equilibrium; NA, not applicable.

In the case of rs689466, the TT genotype was predominant, whereas the CC genotype appeared with the lowest frequency in both cohorts. Interestingly, a significant difference in genotype distribution between the T2DM and healthy groups was found for rs20417. The CC genotype was the most prevalent in both groups, representing 67.33% of the T2DM group and 56.44% of the healthy controls. Notably, the GG genotype was observed in only 2.97% of the T2DM group compared with 12.87% in the healthy control group, suggesting a potential association with disease status.

Subsequently, the allele frequencies of the three *COX-2* SNPs were analyzed, as presented in Table III. No statistically significant differences were observed for rs2066826 and rs689466 (P>0.05). For rs689466, the T allele was predominant in both groups, accounting for 90.10% of all alleles,

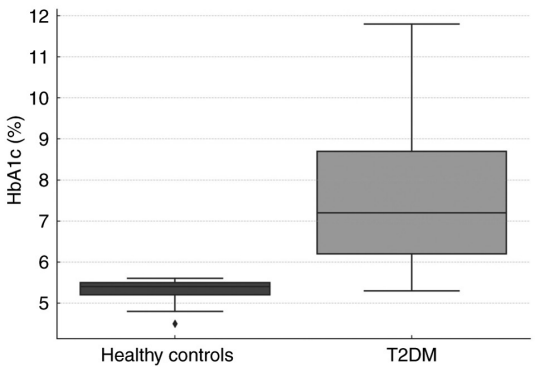

Figure 1. HbA1c levels in healthy controls and patients with T2DM. The median for the control group is 5.4 (IQR: 5.2-5.5%) compared to 7.2 (IQR: 6.2-8.7%) for T2DM. HbA1c, hemoglobin A1c; T2DM, type 2 diabetes mellitus, IQR, interquartile range.

Table IV. Haplotype frequency of cyclooxygenase-2 loci in healthy control and T2DM groups.

rs689466	rs20417	rs2066826	T2DM	Healthy controls	OR (95% CI)	P-value
T	C	C	142 (70.30)	123 (60.89)	1.52 (1.01-2.30)	0.046
T	G	T	25 (12.38)	38 (18.81)	0.61 (0.35-1.05)	0.074
C	C	C	20 (9.90)	18 (8.91)	1.12 (0.58-2.19)	0.733
T	G	C	11 (5.45)	17 (8.42)	0.63 (0.29-1.37)	0.239

Haplotype frequencies are calculated per chromosome (two per individual), resulting in 202 haplotypes per group. Data are presented as n (%). OR, odds ratio; T2DM, type 2 diabetes mellitus.

Table V. Multivariate logistic regression analysis on the association of key variables with the risk for type 2 diabetes mellitus.

Variable	OR	95% CI	P-value
Age (≥60 vs. <60 years)	0.82	0.38-1.78	0.6224
Sex (female vs. male)	1.08	0.58-2.03	0.8051
BMI (≥25 vs. <25)	1.09	0.40-2.95	0.8645
HTN (yes vs. no)	4.44	2.37-8.29	<0.0001
Triglycerides (high vs. normal)	2.21	1.18-4.11	0.0119
rs20417 (vs. GG)			
CG	5.09	1.18-21.96	0.0290
CC	5.39	1.34-21.72	0.0178

BMI, body mass index; HTN, hypertension; OR, odds ratio.

while the C allele was relatively rare, comprising only 9.90%. Similarly, for rs2066826, the C allele was the most common in both patients with T2DM (85.64%) and healthy controls (79.21%), while the T allele was present at a frequency of 14.36 and 20.79%, respectively. Although the association between rs2066826 and T2DM did not reach statistical significance, a P-value of ~0.1 suggests a potential trend toward significance and may warrant further investigation.

Association between COX-2 genotypes and biochemical parameters in patients with T2DM. To explore whether genetic variation in the *COX-2* gene may influence metabolic or kidney-related traits in individuals with T2DM, the association between the three SNPs and biochemical parameters within the T2DM group was examined (Table SI). The biochemical parameters included serum markers of glycemic control (HbA1c), lipid metabolism [triglycerides, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL)] and renal function (creatinine and urea).

The analysis revealed no statistically significant associations between any of the examined SNP genotypes and the biochemical measures (all P>0.05). These results suggest that, in the present study, *COX-2* genetic variants were not major contributors to variations in metabolic or renal profiles among individuals with T2DM.

Haplotype analysis. To explore whether combinations of alleles across the three SNPs in the *COX-2* gene may be associated with T2DM, a haplotype analysis was conducted using the SHEsisPlus software platform. This approach aimed to identify potential genomic blocks that may confer increased susceptibility to or protection against T2DM.

As shown in Table IV, the most common haplotype identified, T-C-C, was significantly more frequent among patients with T2DM (70.30%) than healthy controls (60.89%). This haplotype was associated with a 52% increased risk of T2DM (OR=1.52; 95% CI: 1.01-2.30; P=0.046), suggesting a potential risk allele combination within the *COX-2* gene.

By contrast, the T-G-T haplotype appeared more frequently in healthy controls (18.81%) than in patients with T2DM (12.38%), with an OR of 0.61 (95% CI: 0.35-1.05; P=0.074). Although this trend suggests a possible protective effect of this haplotype against T2DM, the result did not reach statistical significance.

Together, these findings highlighted the potential relevance of *COX-2* haplotypes in modulating diabetes risk and warrant further investigation in larger, independent cohorts.

Multivariate logistic regression analysis of key demographic, clinical and genetic factors associated with T2DM. To evaluate the independent contribution of demographic, clinical and genetic factors to the risk of T2DM, a multivariate logistic regression analysis was performed (Table V). The analysis showed that hypertension was strongly associated with T2DM, with hypertensive individuals being more than four times more likely to develop the disease compared with healthy controls (OR=4.44; 95% CI: 2.37-8.29; P<0.0001). Similarly, individuals with high triglyceride levels exhibited a significantly

increased likelihood of developing T2DM (OR=2.21; 95% CI: 1.18-4.11; P=0.0119). By contrast, age, sex and BMI did not show statistically significant associations with T2DM in this model.

Notably, the *COX-2* rs20417 SNP remained significantly associated with T2DM after adjustment for other covariates. Individuals with the CG genotype were nearly five times more likely to develop T2DM (OR=5.09; 95% CI: 1.18-21.96; P=0.0290), and those with the CC genotype exhibited a similarly elevated risk (OR=5.39; 95% CI: 1.34-21.72; P=0.0178).

Discussion

The findings of the present study suggested a possible association between the *COX-2* SNP rs20417 and the risk of T2DM. The CC genotype and C allele were more frequent among individuals with T2DM, suggesting a possible risk-enhancing effect. Conversely, the GG genotype and G allele were more prevalent in the control group, suggesting a potential protective effect against T2DM. By contrast, the other two investigated *COX-2* polymorphisms, rs689466 and rs2066826, did not show a statistically significant association with T2DM in the Jordanian population.

COX-2 is an inducible enzyme that has been shown to play a central role in inflammatory responses and has been implicated in the development of T2DM (19,23). Experimental studies have shown that COX-2-stimulated PGE2 contributes to β-cell inflammation and impaired insulin secretion under chronic inflammatory conditions, such as cytokine-mediated pancreatic stress (24,25). In addition, genetic variation in inflammatory genes, including *COX-2*, has been associated with metabolic traits and diabetes susceptibility in diverse populations, although the associated polymorphisms vary by ethnicity (18,19). Such heterogeneity may reflect differences in the genetic background, allele frequencies, environmental exposures and gene-environment interactions.

Regarding rs689466, the present study did not reveal any significant association with T2DM, consistent with findings from previous studies conducted in different populations (18,19). Similarly, the rs2066826 did not show a statistically significant association with T2DM in the Jordanian population. However, this contrasts with the results of Konheim and Wolford (19), who reported a significant association between this SNP and T2DM in Pima Indians. Additionally, Rudock *et al* (26) found that the A allele of rs2066826 was markedly associated with lower levels of coronary calcified plaques while the AA genotype was associated with increased levels of carotid calcified plaques in individuals with diabetes, highlighting the potential relevance of this SNP in cardiovascular risk modulation within populations with diabetes. Notably, in the present analysis, the P-value for rs2066826 allele frequency was closer to the conventional threshold for statistical significance (P=0.1163 approaches the conventional threshold for statistical significance P<0.05), suggesting that a larger sample size may reveal a meaningful association of this SNP with the onset of T2DM. Accordingly, the rs2066826 polymorphism may warrant further investigation in expanded or multi-center studies to better understand its potential role in T2DM susceptibility and related cardiovascular complications.

The association of rs20417 with diabetes risk may be linked to its involvement in inflammatory and metabolic regulatory pathways, consistent with prior findings on *COX-2* gene variants. Konheim and Wolford (19) identified both rs20417 and rs2066826 as being associated with T2DM in the Pima Indian population. In their study, the G allele of rs20417 was predominant, accounting for 88% of the population, while the C allele was associated with an increased risk of T2DM. In line with this, Churm *et al* (23), who investigated this SNP in patients from the University College London Diabetes and Cardiovascular Study group, reported that while the G allele remained the major allele, the C allele was markedly associated with T2DM, angina and reduced HDL levels. However, a study in the Bai ethnic group of China reported a lower frequency of the G allele compared to that of the C allele for the rs20417 SNP in healthy individuals (27). These population-level variations in allele distribution may be influenced by several factors, including ethnic background, genetic drift, natural selection and environmental exposures, which collectively shape allele and genotype frequencies over time.

In the present Jordanian cohort, the C allele was found to be the most prevalent variant of the rs20417 polymorphism, accounting for 76.98% [(166+145)/404 alleles x 100%)] of the total population, with a significantly higher frequency among individuals with T2DM. However, unlike the findings of Churm *et al* (23), the current findings showed no significant association between rs20417 and individual lipid profile parameters, including HDL, LDL, total cholesterol and triglycerides. Despite differences in overall allele distribution, these findings consistently support a T2DM risk-enhancing role for the C allele across multiple populations.

Another study investigated the association of COX-2 gene variants with early indicators of cardiovascular disease in a population with T2DM. The findings revealed that individuals carrying the CC genotype at rs20417 exhibited significantly increased levels of carotid calcified plaques (26). This reinforces the hypothesis that the C allele of rs20417 may contribute not only to diabetes susceptibility but also to its cardiovascular complications, underscoring the clinical importance of this variant in metabolic and vascular health. However, it is worth noting that another study found that the minor allele of rs20417 is associated with a reduced risk of major adverse events from cardiovascular diseases (28).

The rs20417 promoter variant in the *COX-2* gene has been shown to reduce promoter activity and lower C-reactive protein levels under inflammatory states, suggesting a regulatory role in inflammation (29). This SNP was suggested to alter the number of CpG islands present within the promoter of the *COX-2* gene and, therefore, may affect its expression level (30). The precise mechanism by which rs20417 reduces *COX-2* promoter activity has not yet been fully elucidated. However, this SNP resides within a potential specificity protein 1 (Sp1) binding site within the *COX-2* promoter region, suggesting that it may alter Sp1 transcription factor binding and consequently affect gene expression regulation (29,31). The present findings suggested that the C allele is associated with an increased risk of T2DM, supporting the notion that altered *COX-2* expression may contribute to metabolic dysregulation in diabetes.

The potential involvement of COX-2 in the pathogenesis of T2DM is further supported by evidence of its abnormal expression in pancreatic islets of individuals with diabetes. Pancreatic islets continually and dominantly express the

inducible COX form, COX-2 (32), and a previous study demonstrated elevated *COX-2* expression in the islets of organ donors with T2DM compared with those of non-diabetic organ donors (13). The same study indicated that upregulated *COX-2* expression in human T2DM islets is associated with inflammatory cytokines and may contribute, at least in part, to β-cell dysfunction. These findings provide functional insights that complement the current genetic results, reinforcing the notion that *COX-2* variants such as rs20417 may influence T2DM susceptibility through both inflammatory signaling pathways and pancreatic islet impairment.

The haplotype analysis of the present study revealed that the T-C-C haplotype, comprising alleles from rs689466, rs20417 and rs2066826, respectively, was significantly more frequent among individuals with T2DM (70.30%) compared with healthy controls (60.89%). This haplotype was associated with an OR of 1.52, meaning that individuals carrying the T-C-C haplotype exhibited a 52% higher likelihood of developing T2DM compared with those without this haplotype. Although modest, this elevated OR suggested a potential risk-enhancing genetic profile, supporting the role of *COX-2* gene variants in contributing to diabetes susceptibility.

The present study has certain limitations that should be acknowledged. First, a formal power calculation was not performed prior to the study. Post-hoc power analysis indicated moderate power (70%) for detecting the observed rs20417 allelic association. However, power was limited for detecting associations with rare genotypes such as rs2066826 TT (21%) due to low minor allele frequencies. This may have reduced the ability to detect true associations for some variants. Second, while correction for multiple comparisons (three SNPs tested for genotype and allele associations plus haplotype analysis) was not applied due to the pre-specified, literature-based selection of variants, future studies with larger samples and independent validation will be valuable in confirming these associations.

In conclusion, the present study provided evidence that the *COX-2* rs20417 polymorphism is significantly associated with increased risk for T2DM in the Jordanian population, independently of traditional clinical risk factors. The C allele and CC genotype were more frequent among patients with diabetes and may contribute to disease susceptibility through inflammatory and metabolic regulatory pathways. No significant associations were observed for rs689466 or rs2066826, although the latter showed a trend toward significance, warranting further investigation in larger or multi-center cohorts. These findings highlight the potential of *COX-2* genetic variants as biomarkers for T2DM risk and underscore the potential relevance of genetic variants in risk stratification.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

NA was involved in study conception, formal data analysis, funding acquisition, investigation of the genetic association analysis, project administration, supervision and writing the original draft. LE was involved in study conception, investigation of the genetic association analysis, project administration and supervision. SF was involved in data curation, methodology (performing experiments) and writing the original draft. KM was involved in data curation and methodology (performing experiments). NS conceived the study and performed data curation and clinical interpretation of data and results. NA and LE confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board (IRB) of Jordan University of Science and Technology and King Abdullah University Hospital (approval no. 47/163/2023; Irbid, Jordan), under the oversight of the Deanship of Research at Jordan University of Science and Technology. All participants provided written informed consent before being enrolled in the study, confirming their voluntary participation.

Patient consent for publication

All participants were informed about the study's objectives, assured that their data would be de-identified to protect their privacy, and provided written informed consent for the use of their blood samples for scientific research purposes.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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