Original Article

Genetic Patterns in LDLR Gene Polymorphism among Individuals with Co-Existing Diabetes and Coronary Artery Disease

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Abstract

Background: This research investigates the association between genetic polymorphism in the low-density lipoprotein receptor (LDLR) gene and type 2 diabetes mellitus (T2DM) with coronary artery disease (CAD). It examines two single nucleotide polymorphisms (SNPs), rs5925 (C/T), and rs11669576 (A/G), located within the LDLR gene on chromosome 19. The focus of the study involves analyzing the genotypes and haplotypes associated with these two SNPs. **Objective:** To comprehend the influence of the two SNPs and their combined presence in haplotypes on the onset of CAD in individuals with diabetes. **Materials and Methods:** This research involved 400 participants divided into two groups: 200 individuals with T2DM and CAD and 200 unaffected individuals. Logistic regression analysis was used to assess the risk associated with LDLR gene polymorphisms and haplotypes. Genotyping employed the allele discrimination/SNP's real-time PCR TaqMan method. **Results:** The study revealed a significant association between rs5925 T > C polymorphism and an increased risk of T2DM and CAD, independent of age, sex, and body mass index. The presence of the C allele significantly elevated the risk. Haplotypes GC and CT were found to be strongly associated with the diseases. GC increased the risk of T2DM and CAD, while CT appeared to be protective. Haplotype AT did not show a significant association. Linkage disequilibrium analysis indicated a moderate level of association (D' = 0.74) and a weak level of association (r^2 = 0.03) between the two SNPs, suggesting partial correlation. **Conclusion:** The study highlights the role of genetic variations in the LDLR gene, particularly SNP rs5925, in the development of T2DM and CAD. Haplotype analysis revealed significant association, with GC increasing risk and CT showing protective effects, while haplotype AT had no substantial association with the diseases.

Keywords: Allele, genotype, haplotype, LDLR, linkage disequilibrium, SNPs

Introduction

Diabetes mellitus (DM) and coronary artery disease (CAD) are complex multifactorial diseases with significant genetic components.[1,2] Understanding the genetic factors contributing to the comorbidity of DM and CAD is crucial for improved risk assessment, prevention, and treatment strategies.[3,4]

The rise in type 2 diabetes (T2DM) has been swift, primarily driven by the growing average lifespan, a surge in obesity rates, and the adoption of Westernized lifestyles in developing nations.[5,6] Nevertheless, the primary contributors to sickness and death in T2DM are the enduring complications it brings about.[7] T2DM stands as a significant standalone contributor to cardiovascular disease (CVD), which itself constitutes a prominent global health challenge. Research has demonstrated that individuals with diabetes are at a significantly higher risk, approximately two to four times

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more likely, to develop CAD and experience myocardial infarction (MI).[8]

Until now, several studies have showcased how the interplay between T2DM and cardiovascular risk fosters a step-by-step advancement of vascular damage, ultimately culminating in the development of atherosclerosis.[9]

Numerous additional risk factors for CVD have been pinpointed, including dyslipidemia, oxidative stress, smoking, alcohol consumption, and genetic factors.[10] Dyslipidemia, characterized by lipoprotein irregularities, can exacerbate both microvascular and macrovascular complications while also contributing to the development of atherosclerosis in individuals with T2DM.[11]

Among the genes implicated in this complex interplay is the low-density lipoprotein receptor (LDLR) gene, with specific single nucleotide polymorphisms (SNPs) such as rs5925 and rs11669576 gaining attention for their potential roles in the development of T2DM with CAD The LDLR is located on chromosome 19 (19p13.2), is 45 kb long.[12]

LDLR has seven functional domains: the promoter translation signal sequence (exon 1), the ligand binding domain (exons 2–6), the epidermal growth factor precursor homology domain (exons 7–14), the O linked sugar domain (exon 15), the membrane spanning domain (exons 16–17), and the cytoplasmic domain (exons 17–18).[13]

The genetic variation rs11669576 in exon 8 involves coding sequence variance (in the plus strand) [GCC] > [ACC] leading to a *missense* variant [alanine/threonine change] at amino acid 370. On the other hand, the genetic variation rs5925 in exon 13 involves coding sequence variance (in the plus strand) [GTT] > [GTC] leading to *synonymous (silent*) variant (Valine) > (Valine) (i.e., no change). These two genetic variations within the internalization EGF precursor homology section of LDLR.[14]

It has been suggested that genetic variations in LDLR could be strongly linked to the advancement and development of specific cerebrovascular conditions, like cerebral infarction.[15] LDLR is known to have a significant role in maintaining cholesterol balance, and mutations in its genetic makeup could potentially result in lowered LDLR expression. This, in turn, may cause increased LDL levels, impaired receptor function, familial hypercholesterolemia, and ultimately contribute to the onset of cerebral infarction.[16]

One or two of the genetic variations (rs5925) may lead to damage in the transport or metabolism of low-density lipoprotein (LDL).[17] LDLR functions as a glycoprotein spanning the cell membrane on hepatocytes, holding a significant responsibility in maintaining balanced cholesterol levels within the body.[18] The LDLR gene encodes the LDLR, a critical player in lipid metabolism.[19] It functions to remove low-density lipoprotein cholesterol (LDL-C) from the bloodstream. Dysregulation of LDLR can lead to elevated LDL-C levels, a known risk factor for CAD.[20]

Furthermore, variations within the LDLR gene, particularly SNPs rs5925 and rs11669576, have been examined for their potential roles in T2DM with CAD. A linkage of familial hypercholesterolemia has been established with a total of 1700 mutations found within the LDLR gene.[21] Defects or changes in this gene result in the genetic condition known as familial hypercholesterolemia, which is inherited in an autosomal dominant manner.[20]

Additionally, alternative splicing leads to the creation of various transcript variants. This stands as a conventional risk element for cardiovascular disease.[22] In this research, we examined LDLR haplotypes in the population change over time and explored their connection to the development of CAD.[21]

Materials and methods

Subjects

The research adopted a hospital-centric methodology, where patient samples were procured from the catheterization unit situated in Al-Najaf Center for Cardiac Surgery and Transcatheter Therapy, located within Al-Sadr Medical City in Najaf city. Data collection occurred from November 2021 to March 2022, with all clinical diagnoses conducted at this facility and blood samples collected in the clinic's laboratory.

Weight and height were recorded and used to calculate the body mass index (BMI). Obesity was defined as having a BMI of 25 kg/m² or higher, following the criteria established by the World Health Organization (WHO).[23]

Hypertension was diagnosed when blood pressure readings exceeded 140/90 mm Hg or if participants were taking antihypertensive medications. The diagnosis of T2DM cases followed the standards set by the American Diabetes Association (ADA), with fasting blood glucose (FBG) levels ≥126 mg/dL and hemoglobin A1C (HbA1C) levels ≥6.5%.[23]

Dyslipidemia was characterized by total cholesterol (TC) levels above 200 mg/dL, triglycerides (TG) levels over 150 mg/dL, LDL levels exceeding 130 mg/dL, and high-density lipoprotein (HDL) levels below 40 mg/dL for males and under 50 mg/dL for female.[24]

Individuals were confirmed to have CAD through coronary angiography, which revealed at least a 70% stenosis in a major coronary artery or one of its branches.[25]

LDLR genotyping

The LDLR genotyping processed by extracting genomic DNA (gDNA) from whole blood samples using purification kits (Add Prep Genomic Product Code: 10023, Korea). The quality of the DNA was evaluated by employing

a Bio-drop (Biodrop Ltd., Cambridge, UK) at 260 and 280 nm wavelengths. Integrity was assessed on agarose gel electrophoresis.

The Mx3005P qPCR system was used to genotype the alleles. It is high-performance optical system, precise temperature control, intuitive software interface, and compatibility with a wide range of fluorescent dyes and probes. These features combine to deliver accurate and reliable qPCR results with enhanced efficiency.[26]

The genotyping process involved the use of specific primers and probes for two variants, both are designed according to the National Center for Biotechnology Information (NCBI); rs11669576 G>A and rs5925 T>C. For rs11669576, the forward (F) primer was TTCCCGTGCTCACCCA, the reverse (R) primer was TGGCTACAAGTGCCAGTGT, and the corresponding probes were FAM-TGCAGGCTTCGTG-BHQ for the wild allele and HEX-CTTGCAGGTCTTCGTG-BHQ for the mutant allele. Similarly, for rs5925, the forward (F) primer was CCTTACCTCTTGGCTGGGT, the reverse (R) primer was CCTCACAGGTTCCGATGTCAA, and the corresponding probes were FAM-TTGTGGAAGAGAACCATATC-BHQ for the wild type and HEX-TTGTGGAAGAGGACCATATC-BHQ for the mutant type.

In each allele discrimination/SNP's real-time PCR reaction, the components included GoTaq probe qPCR Master Mix (12.0 µL), forward primer (1 µL), reverse primer (1 µL), wild probe (1 µL), mutant probe (1 µL), genomic DNA (4 µL), and nuclease-free water (5 µL), resulting in a total volume of 25 µL. The real-time PCR reactions consisted of three primary steps in each cycle and typically ran for 40 cycles. For rs11669576, the initial denaturation conditions were 95°C for 5 min in one cycle. Subsequently, denaturation occurred at 95°C for 10 s in 40 cycles, followed by annealing at 58°C for 1 min in 40 cycles, and an extension at 72°C for 30 s in 40 cycles.

For rs5925, the initial denaturation conditions were set at 95°C for 5 min in one cycle. The denaturation step conditions comprised 95°C for 20 s in 40 cycles, followed by annealing at 60°C for 50 s in 40 cycles, and an extension at 72°C for 30 s in 40 cycles. This experimental approach utilized fluorogenic probes labeled with distinct dyes, Hex and FAM, to differentiate alleles differing by a single nucleotide.

The presence or absence of a specific allele was determined by analyzing the CT value obtained for the allele-specific probe. Amplification plots in the results section illustrate the relationship between cycles and fluorescence for specific data collection points.[27]

Taq polymerase plays a crucial role in distinguishing between various alleles as it binds to specific fluorogenic probes on the template, causing the probes to be cleaved, thus releasing a previously obscured reporter dye, known as "FAM or Hex." For each assay, we required two PCR primers without labels and two allele-specific probes, as noted by Heist *et al*.[27]

To determine the genotype of an unknown DNA sample, fluorescence was assessed in comparison to the background fluorescence levels of both dyes. When the fluorescence significantly exceeded the background for the dye associated with the wild-type allele but not for the dye linked to the mutant allele, the sample was classified as wild-type homozygous. Conversely, when the fluorescence surpassed the background for the dye identifying the mutant allele but not for the dye identifying the wild-type allele, the sample was categorized as mutant homozygous. In cases where the sample displayed intermediate fluorescence values for both dyes, it was designated as heterozygous for both alleles.[28]

Ethics statement

1. Authorization (Number 2977, dated May 19, 2022) was obtained from the scientific committee of the Biochemistry Department at the University of Kufa, Iraq.
2. Consent was acquired from the Al-Najaf Health Directorate, a division of the Ministry of Health, and from the Information Center for Research & Development of Najaf Province (Number 24848, dated June 16, 2022).
3. All participants were briefed on the study's objectives and procedures, and their verbal consent was sought for their involvement.

Statistical analysis

By using various inheritance models, including recessive, codominant, dominant, additive, and allelic models (specific details have been omitted here), a comprehensive analysis was executed through the application of multi-nominal logistic regression analysis, utilizing IBM SPSS Version 25.0 software. The outcomes were presented in terms of *P* value, odds ratios (OR), and 95% confidence intervals (CI). To ascertain whether the population demonstrated genetic equilibrium and to detect any deviations from the expected genetic ratios, the Hardy–Weinberg calculator was utilized. Furthermore, Haplotype analysis was conducted employing software designed for the analysis of haplotype data, version 1.05.

Results

Haplotypes, combinations of alleles on a single chromosome, can provide valuable insights into the genetic basis of complex diseases. Analyzing the haplotype-related patterns of LDLR gene polymorphisms in patients with CAD and diabetes can elucidate their joint effects on disease risk.[29]

Studies have shown that certain LDLR haplotypes are associated with a higher risk of CAD. These haplotypes may exert cumulative effects on lipid metabolism, exacerbating dyslipidemia and increasing CAD susceptibility.[30]

In the context of diabetes, haplotype analysis may reveal synergistic or antagonistic effects between LDLR variants. Investigating these interactions can provide a more comprehensive understanding of the genetic factors contributing to diabetes risk in individuals with CAD.[31]

Assessment of genotype and allele frequencies of LDLR rs11669576

The analysis presents genetic association data for the rs11669576 G > A polymorphism examining various genetic models: codominant, dominant, over dominant, recessive, and additive. The results indicate no significant associations between this polymorphism and T2DM with CAD in most models, except for the recessive model where individuals with the AA genotype exhibit a reduced risk. Moreover, the minor allele frequency (MAF) percentages show no substantial differences between the control and patient groups. The results remained unchanged after adjusting for age, sex, and BMI, suggesting that the presence of the A allele alone is not a significant risk factor for the condition in this particular study population [Table 1].

Assessment of genotype and allele frequencies of LDLR rs5925

Table 2 represents genetic association data for the rs5925 T>C polymorphism, and examines various genetic models. Under the codominant model, both TC and CC genotypes show significantly increased risks for the condition compared to the TT genotype, with substantial OR 3.2 (adjusted), and 10.5 (adjusted), and *P* value of 0.0001. In the dominant model, combining TC and CC genotypes, there is a significant association with an OR of 3.96 (adjusted), along with a *P* value of 0.0001. The over-dominant model also demonstrates a significant association, with an OR of 2.21 (adjusted) and a *P* value of 0.0001. In the recessive model, individuals with the CC genotype exhibit a significantly elevated risk with an OR of 5.7 (adjusted) and a *P* value of 0.0001. The additive model indicates a significant association, with an OR of 3.3 and *P* value of 0.0001. Additionally, the MAF percentages differ significantly between control (20%) and patient (44%) groups, with a *P* value of 0.0001, indicating that the presence of the C allele is strongly associated with an increased risk of the T2DM and CAD in this study population. Overall, the data robustly support a significant and strong association between the rs5925 T>C polymorphism and an elevated risk of the disease conditions across various genetic models. The results remained unchanged after adjusting for age, sex, and BMI.

LDL alleles' linkage disequilibrium analysis

Table 3 that provides a summary of findings derived from a genetic association investigation involving two specific genetic variants, namely rs1169576 and rs5925, within the context of assessing linkage disequilibrium (LD) in LDLR alleles.

The results obtained encompass the following statistical outcomes: Pearson's *P* value for rs1169576, standing at 0.469, and Fisher's *P* value for rs1169576, registering at

Table 1: Assessment of genotype and allele frequencies of LDLR gene rs11669576

rs11669576 G > A	Control 200	Patients 200	Non-adjusted OR (CI 95%)	*P* value	Adjusted OR (CI 95%)	*P* value
Codominant						
GG Wild	164	162	–			
GA	26	36	1.4 (0.81–2.43)	0.23	1.5 (0.85–2.6)	1.64
AA	10	2	0.202 (0.044–0.938)	0.41	0.35 (0.7–1.8)	0.21
Dominant						
GA+AA	36	38	1.11 (0.66–1.84)	0.7	1.3 (0.76–2.2)	0.35
Over dominant						
GG+AA	174	164	–			
GA	26	36	1.4 (0.85–2.5)	0.2	1.43 (0.9–12.7)	0.211
Recessive						
GG+GA wild	190	198	–			
AA	10	2	0.19 (0.04–0.88)	0.03	0.34 (0.07–1.7)	0.2
Additive						
2GG+GA	354	360	–			
2AA+GA	46	40	0.88 (0.55–1.33)	0.5		
MAF % (A allele)	11	10		0.5		

*$P < 0.05$ is considered significant.

Table 2: Assessment of genotype and allele frequencies of LDLR gene rs5925

rs5925 T>C	Control 200	Patients 200	Non-adjusted OR (CI 95%)	P value	Adjusted OR (CI 95%)	*P* value
Codominant						
TT wild T	125	59	–			
TC	67	105	3.3 (2.1–5.1)	0.0001	3.2 (2.03–4.9)	0.0001
CC	8	36	9.5 (4.2–21.7)	0.0001	10.5 (4.5–24.6)	0.0001
Dominant						
TC+CC	75	140	3.9 (2.6–6.01)	0.0001	3.96 (2.6–6.1)	0.0001
Over dominant						
TT+CC	133	95	–			
TC	67	105	2.2 (1.5–3.3)	0.0001	2.21 (1.6–3.37)	0.0001
Recessive						
TT+TC wild	192	165	–			
CC	8	35	5.1 (2.3–11.3)	0.0001	5.7 (2.6–12.9)	0.0001
Additive						
2TT+TC	317	223	–			
2CC+TC	83	177	3.3 (2.22–4.14)	0.0001		
MAF % (C allele)	20	44		0.0001		

*$P < 0.05$ significant

Table 3: LDLR alleles' linkage disequilibrium analysis

SNP	X^2	Pearson's *P*	Fisher's *P*	OR (95% CI)	Allele	G	A
rs1169576	0.469	0.493	0.568	0.855 [0.546–1.338]	Case	360 (0.9)	40 (0.1)
					Control	354 (0.885)	46 (0.115)
rs5925	50.347	$1.29e^{-12}$	$1.47e^{-12}$	3.031 [2.218–4.141]	Allele	T	C
					Case	223 (0.557)	177 (0.442)
					Control	317 (0.792)	83 (0.207)
SNP	X^2	Pearson's P	Fisher's P	OR (95% CI)	Allele	G	A
rs1169576	0.469	0.493	0.568	0.855 [0.546–1.338]	Case	360 (0.9)	40 (0.1)
					Control	354 (0.885)	46 (0.115)
rs5925	50.347	$1.29e^{-12}$	$1.47e^{-12}$	3.031 [2.218–4.141]	Allele	T	C
					Case	223 (0.557)	177 (0.442)
					Control	317 (0.792)	83 (0.207)

0.568. In contrast, Pearson's *P* value for rs5925 is notably low, measuring $1.29e^{-12}$, while Fisher's *P* value for rs5925 is similarly low at $1.47e^{-12}$.

Furthermore, with regard to the OR and the accompanying confidence interval (CI) metrics, the calculated OR for rs1169576 is 0.855, with a corresponding 95% CI spanning from 0.546 to 1.338. Conversely, for rs5925, the OR is notably higher, standing at 3.031, with a 95% CI ranging from 2.218 to 4.141.

Delving deeper into allele frequencies, it is observed that for rs1169576, within cases, allele G exhibits a frequency of 0.9 (equivalent to 90%), whereas allele A is observed at a frequency of 0.1 (or 10%). In the control group, allele G is present at a frequency of 0.885 (88.5%), while allele A is found at a frequency of 0.115 (11.5%). As for rs5925, within cases, allele C is documented with a frequency of 0.442 (44.2%), while allele T is observed at 0.557 (55.7%). In the control group, allele C is noted at a rate of 0.207 (20.7%), and allele T is documented at 0.792 (79.2%).

Regarding rs5925, the computed *P* value strongly indicates a significant association between this specific genetic variant and the presence of T2DM with CAD. The OR of 3.031, accompanied by a 95% CI of [2.218–4.141], signifies that allele C is significantly linked to an increased risk of the disease condition compared to allele T.

In contrast, for rs1169576, the obtained *P* value fail to reach statistical significance. Moreover, the OR and its corresponding CI the value 1, suggesting that a robust association may not exist between this genetic variant and the presence of T2DM with CAD. In summary, the data strongly supports the contention that rs5925 is closely associated with an increased risk of T2DM and CAD, while rs1169576 does not exhibit a significant association with the disease condition.

LDL genotypes' linkage disequilibrium analysis

Table 4, Figures 1 and 2 provide a concise overview of the results derived from a LD investigation focused on

Table 4: LDLR genotypes' linkage disequilibrium analysis

SNP	X^2	Pearson's *P*	Fisher's *P*	Genotype	G/G	G/A	A/A
rs1169576	6.958	0.03	0.028	Case	162 (0.81)	36 (0.18)	2 (0.01)
				Control	164 (0.82)	26 (0.13)	10 (0.05)
rs5925	49.887	$1.47e^{-11}$	$5.85e^{-12}$	Genotype	C/C	T/C	T/T
				Case	36 (0.18)	105 (0.525)	59 (0.295)
				Control	8 (0.04)	67 (0.335)	125 (0.625)

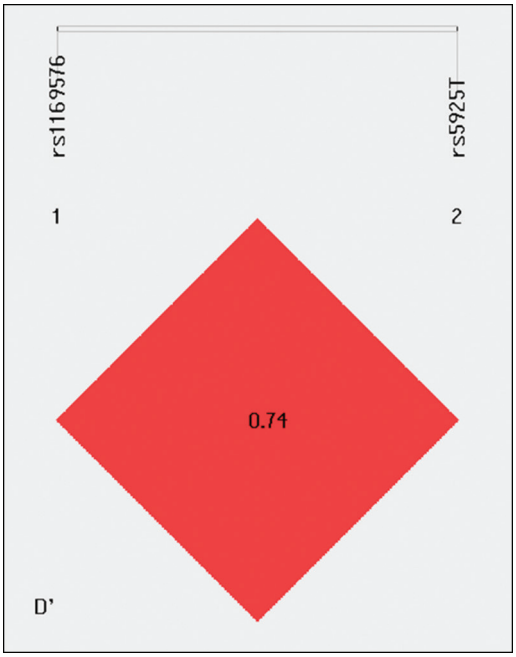

Figure 1: Linkage disequilibrium (LD) D prime (D') non-random association of alleles at different loci rs5925 and rs11669576 of LDLR gene. The value of *D'* = 0.74 333

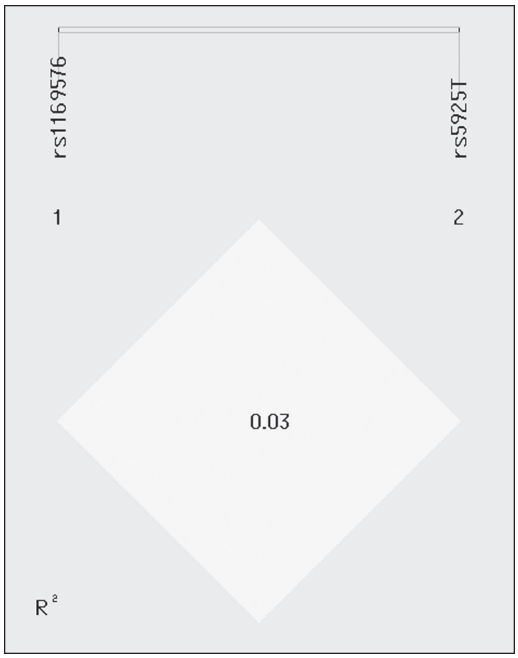

Figure 2: Linkage disequilibrium (LD) *r*-squared (r^2) non-random association of alleles at different loci rs5925 and rs11669576 of LDLR gene. The value of R^2 =0.03

two particular single nucleotide polymorphisms (SNPs), specifically rs1169576 and rs5925.

Concerning rs1169576, the analysis yielded a Pearson's *P* value of 0.03, while the Fisher's *P* value was slightly lower at 0.028. In contrast, rs5925 exhibited notably lower *P* value, with Pearson's at $1.47e^{-11}$ and Fisher's at a more impressive $5.85e^{-12}$. Regarding genotype frequencies, for rs1169576, the case group exhibited a distribution of 0.81 for genotype G/G, 0.18 for G/A, and 0.01 for A/A. In the control group, these proportions were 0.82 for G/G, 0.13 for G/A, and 0.05 for A/A. Transitioning to rs5925, the case group displayed genotype frequencies of 0.18 for C/C, 0.525 for C/T, and 0.295 for T/T. In contrast, the control group manifested genotype frequencies of 0.04 for C/C, 0.335 for C/T, and 0.625 for T/T.

LD analysis fundamentally involves assessing whether alleles at distinct loci exhibit a tendency to be co-inherited more or less frequently than would be anticipated by random chance. Pertaining to rs1169576, both Pearson's and Fisher's *P* value are relatively high ($P < 0.03$ was disregarded), signifying the absence of a statistically significant LD between this SNP and the disease condition.

Consequently, it can be deduced that the genotypes of rs1169576 are not associated with T2DM and CAD. Conversely, for rs5925, the *P* value are exceedingly low, with Pearson's at $1.47e^{-11}$ and Fisher's at $5.85e^{-12}$. This compellingly suggests a substantial LD between rs5925 and the condition, indicating a significant association between the genotypes of rs5925 and the ailment.

In conclusion, the data underscores that rs5925 exhibits statistically significant LD with T2DM and CAD. This implies that the genotypes linked to this SNP may serve as valuable genetic markers or potentially harbor functional implications concerning disease conditions.

LDL haplotype rs1169576 and rs5925 analysis

Table 5 presents a concise summary of results originating from a haplotype analysis, which delves into the combination of alleles at various loci within the context of genetic or clinical outcomes.

In terms of haplotype data, three distinct haplotypes were investigated: GC, CT, and AT. Their respective frequencies were as follows: For haplotype GC, the case group

Table 5: LDLR haplotype rs1169576 and rs5925 analysis haplotype with frequency <0.03 are ignored

Haplotype	Case frequency	Control frequency	X^2	Fisher's *P*	Pearson's *P*	OR (95% CI)
GC	170 (0.425)	83 (0.207)	43.754	$4.49e^{-11}$	$3.72e^{-11}$	2.822 (2.065–3.858)
CT	190 (0.475)	271 (0.677)	33.586	$9.20e^{-9}$	$6.8e^{-9}$	0.43 (0.323–0.573)
AT	33 (0.082)	46 (0.115)	2.373	0.154	0.123	0.691 (0.432–1.107)

exhibited a frequency of 170 (42.5%), while the control group displayed a frequency of 83 (20.7%). For haplotype CT, the case group showcased a frequency of 190 (47.5%), whereas the control group demonstrated a frequency of 271 (67.7%). For haplotype AT, the case group recorded a frequency of 33 (8.2%), and the control group observed a frequency of 46 (11.5%).

OR and CI metrics for these haplotypes were computed as follows: Haplotype GC yielded an OR of 2.822, with a 95% CI spanning from 2.065 to 3.858. Haplotype CT displayed an OR of 0.43, along with a 95% CI ranging from 0.323 to 0.573. Haplotype AT resulted in an OR of 0.691, accompanied by a 95% CI from 0.432 to 1.107.

Haplotype GC was notably more frequent in cases compared to controls. Both Pearson's and Fisher's tests yielded exceedingly low *P* value (specifically, $3.72e^{-11}$ and $4.49e^{-11}$, respectively). Furthermore, the OR of 2.822, combined with a 95% CI that excludes 1, indicates an association between haplotype GC and an elevated risk of T2DM and CAD.

Conversely, haplotype CT displayed a significantly lower frequency in cases than in controls. Once again, both Pearson's and Fisher's tests resulted in very low *P* value ($6.8e^{-9}$ and $9.20e^{-9}$, respectively). The OR of 0.43, coupled with a 95% CI below 1, implies that haplotype CT is linked to a reduced risk of the condition, signifying a protective effect. As for haplotype AT, it did not exhibit a substantial difference in frequency between cases and controls. The *P* value from both Pearson's and Fisher's tests were relatively high (0.123 and 0.154, respectively), and the CI of the OR included 1. This suggests that haplotype AT is not strongly associated with development of disease conditions.

In conclusion, the haplotype analysis underscores significant associations between haplotypes GC and CT and the studied condition. Haplotype GC is associated with an increased risk, while haplotype CT appears to offer protection. In contrast, haplotype AT does not demonstrate a significant association with the condition based on the available data.

Discussion

In this study, the rs5925 polymorphism is a type of genetic alteration located in exon 13 of the coding sequence. This polymorphism doesn't affect the translation process, as the amino acid remains the same (Valine (Val) > Valine (Val)). However, it has been found to influence the splicing efficiency of the LDLR gene.[32]

Moreover, the rs5925 polymorphism has been anticipated to be among the SNPs responsible for inducing familial hypercholesterolemia within the Malaysian population.[33] Conversely, the present investigation stands in opposition to a study conducted in India that examined the influence of specific LDLR SNPs, one of which includes the rs5925.[34]

Additionally, this study explored the rs11669576 LDLR gene polymorphism under various inheritance models and found that no link with T2DM and CAD. This may be due to its common occurrence, normal protein function, and absence of disease associations. It's the first study to establish this negative link.

A 2017 study by Martinelli *et al.*[35] found significant associations between LDLR haplotypes and CAD risk in a European population.

Comparable studies have also indicated that interactions between LDLR variants and genes related to diabetes can exert an influence on the susceptibility to CAD in diabetes.[36]

On the other hand, the analysis of LDLR allele LD, particularly concerning rs1169576 and rs5925, reveals distinct relationships with the coexistence of T2DM and CAD. The significantly low *P* value and the high OR for rs5925 indicate a strong LD and a substantial association with the diseases.[37]

These findings are consistent with a 2019 study by Jha and colleagues, which identified rs5925 as a robust candidate for genetic susceptibility to the co-occurrence of T2DM and CAD.[34]

Individuals with both heterozygous (GA) and homozygous (AA) genotypes were not significantly associated with these diseases. Thus, these results suggest that the rs11669576 SNP does not contribute to the susceptibility to T2DM and CAD in the Iraqi population.

Table 5 summarizes findings from haplotype analysis, focusing on three haplotypes: GC, CT, and AT. In the case group, GC was 42.5%, CT was 47.5%, and AT was 8.2%. In the control group, GC was 20.7%, CT was 67.7%, and AT was 11.5%.

OR and CICI for these haplotypes: GC had an OR of 2.822 (95% CI: 2.065–3.858). CT had an OR of 0.43 (95% CI: 0.323–0.573). AT had an OR of 0.691 (95% CI: 0.432–1.107).

Haplotype GC was notably more frequent in cases compared to controls. Both Pearson's and Fisher's tests yielded exceedingly low *P* value (specifically, $3.72e^{-11}$ and $4.49e^{-11}$, respectively). Furthermore, the OR of 2.822, combined with a 95% CI that excludes 1, indicates an association between haplotype GC and an elevated risk of T2DM and CAD.

Conversely, haplotype CT displayed a significantly lower frequency in cases than in controls. Once again, both Pearson's and Fisher's tests resulted in very low *P* value ($6.8e^{-9}$ and $9.20e^{-9}$, respectively). The OR of 0.43, coupled with a 95% CI below 1, implies that haplotype CT is linked to a reduced risk of the condition, signifying a protective effect. As for haplotype AT, it did not exhibit a substantial difference in frequency between cases and controls.

The haplotype analysis reveals significant associations between GC and CT with the studied condition. GC is linked to increased risk, while CT seems protective. However, haplotype AT is not significantly associated. Additionally, the linkage disequilibrium (LD) D prime (D') for alleles at rs5925 and rs11669576 in the LDLR gene is 0.74, indicating a moderate level of non-random association, implying some correlation in their inheritance.[38]

Furthermore, in the context of non-random associations between alleles at different loci, the LD *r*-squared (r^2) for rs5925 and rs11669576 within the LDLR gene was found to be $R^2 = 0.03$ (as depicted in Figure 2). This value signifies a weak or low level of non-random association between alleles at the rs5925 and rs11669576 loci within the LDLR gene. This suggests that these alleles are predominantly inherited independently of each other.[39]

Conclusion

1. rs5925 variant linked to increased increasing risk of T2DM and CAD.
2. No association of rs11669576 variant with increasing risk of T2DM and CAD.
3. GC haplotype increases risk, CT haplotype is protective.
4. Moderate independence between the two SNPs.

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Conflicts of interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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