Biochemistry and Biophysics Reports 42 (2025) 101992

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Deciphering the association of cholesteryl ester transfer protein (CETP) gene polymorphisms with high-density lipoprotein cholesterol (HDL-c) levels in the Bangladeshi population

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ARTICLE INFO

Keywords:
CETP gene polymorphism
Hypoalphalipoproteinemia
CETP -629C/A
CETP 277C/T
rs1800775
rs708272
HDL cholesterol
Bangladeshi population

ABSTRACT

Cholesteryl ester transfer protein (CETP) gene polymorphisms influence CETP expression and high-density lipoprotein cholesterol (HDL-c) levels, yet their genetic impact remains unexplored in the Bangladeshi population, where low HDL-c is prevalent. This study examined the association of CETP −629C/A and 277C/T polymorphisms with circulating HDL-c levels in 402 individuals (217 males, 185 females). Serum lipid profiles were measured using an automated analyzer, and CETP polymorphisms were genotyped using PCR-RFLP. The −629C/A and 277C/T polymorphisms were in Hardy–Weinberg equilibrium, with heterozygous genotypes being the most frequent. While −629C/A genotypes showed no significant difference between the high and low HDL-c groups, individuals carrying the −629AA and CA + AA genotypes had significantly higher HDL-c levels compared to CC carriers ($p = 0.023$, $p = 0.043$). For the 277C/T, TT genotype differed significantly between the high and low HDL-c groups ($p = 0.011$, OR = 0.37) and, individuals carrying the 277 TT and CT + TT genotypes had significantly higher HDL-c compared to the CC genotype ($p = 0.002$, $p = 0.019$). Additionally, allelic analysis suggested a marginal association between the 277T allele and increased HDL-c levels ($p = 0.051$, OR = 0.59). Multiple regression analysis confirmed an inverse association between −629CC ($\beta = -1.106$, $p = 0.038$) and 277CC + CT ($\beta = -0.963$, $p = 0.016$) with HDL-c levels. However, no significant differences were observed in total cholesterol, triglycerides, LDL-c, or apolipoprotein levels across genotypes. These findings suggest that CETP −629CC, 277CC, and CT genotypes contribute to low HDL-c levels in the Bangladeshi population, highlighting the potential role of CETP genetic screening as a biomarker for identifying individuals at risk of HDL-c deficiency and associated cardiovascular complications.

1. Introduction

The most significant and well-established risk factor for cardiovascular disease (CVD) is hypoalphalipoproteinemia (HA), which raises mortality and morbidity [1–3]. Bangladesh and other low- and middle-income nations have higher rates of mortality from CVD [4,5]. The pathophysiology of coronary artery disease (CAD) in Bangladesh may be influenced by dyslipidemia, particularly HA, or low levels of high-density lipoprotein cholesterol (HDL-c), as the risk of CVD is higher in South Asian populations than in Western ones [6,7]. Low levels of HDL-c have been observed in approximately 70–95 % of people [8–10] and are the most common lipoprotein/lipid abnormality in the Bangladeshi rural population as well as in the urban population [11–14]. The cardioprotective function of HDL is postulated to be reverse cholesterol transport (RCT) [15,16]; however, other functions, including nitric oxide promotion and endothelial function enhancement [17] and antioxidative, anti-inflammatory [18], antithrombotic [17] and antiapoptotic [19] effects, have a profound impact on the vascular system.

The plasma protein known as cholesteryl ester transfer protein (CETP) has gained attention recently as a key modulator of the metabolism of high-density lipoprotein cholesterol (HDL-c), which has a major effect on lifespan and healthy aging [20–22]. 476 amino acids are encoded by the CETP gene, which is found on 16q12–21. CETP is a

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https://doi.org/10.1016/j.bbrep.2025.101992
Received 23 January 2025; Received in revised form 16 March 2025; Accepted 25 March 2025

hydrophobic glycoprotein primarily located in the plasma, where it facilitates the transfer of cholesteryl esters between lipoproteins [23]. In RCT, CETP is primarily engaged in moving cholesterol to the liver for removal from peripheral tissues [23].

It exchanges cholesteryl ester (CE) from HDL-c for triglycerides (TGs) from TG-rich lipoproteins (TGRLs), such as very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) [23–25]. In this way, CETP influences the triglyceride content of TGRL lipoproteins and significantly affects HDL-c levels. Several genetic mutations of the CETP gene cause its deficiency, and these mutations are associated with higher plasma HDL-c levels [26–28], and single-nucleotide polymorphisms (SNPs) in the CETP gene were found to influence the circulating CETP mass and HDL-c in different populations studied [29–34]. Since HA is very common among the general population of Bangladesh, we chose two common SNPs that were previously studied in other populations and found to be associated with HDL-c concentration [29–34]. The CETP −629C/A (rs1800775) and 277C/T (rs708272) SNPs are common variations of the CETP gene and influence HDL-c levels in different populations [29,32].

The A allele (variant) of the −629C/A polymorphism results in increased HDL-c and lower CETP production when compared to the C allele [30,33–35]. In Singapore's multiethnic population, however, there is no correlation between HDL-c and −629C/A transversion [32]. The most researched SNP is TaqIB (rs708272), which is found at nucleotide 277 of intron 1 (277C/T). The TaqI restriction enzyme identifies the mutation at this location, leading to the development of three genotypes: B1B1 (CC), B1B2 (CT), and B2B2 (TT) [29,36]. Although other studies found no correlation between the C/T transition and serum HDL-c [37] or sex-based relationships exclusively for males [38], this transition results in a decrease in circulating CETP activity and an increase in HDL-c [31,35,39,40]. Several studies have been conducted on the Asian population, but few have been conducted in South Asia [38,41] or in the people of Bangladesh. Thus, studying this genetic susceptibility in our population is highly important.

2. Methods and materials

2.1. Study groups

In this cross-sectional study, 402 unrelated healthy Bangladeshi adults (aged 20–65 years) were purposively included from various regions of Bangladesh (Mirpur −1, staff of the Bangladesh University of Health Sciences; Ashulia, Savar; Joydevpur, Gazipur; Karar Char, Narsingdi). The study comprised 217 males (54 %) and 185 females (46 %), all without a history of diabetes, hypertension, chronic kidney disease (CKD), familial hypercholesterolemia, hypothyroidism, or other serious comorbid conditions such as infections, stroke, myocardial infarction, or major surgery. Subjects with a history of using drugs that significantly affect glucose metabolism (antihyperglycemic agents, glucocorticoids, thiazide diuretics, etc.), lipid metabolism, hormone therapy or lactation and pregnant women were excluded.

2.2. Sample size calculation

The minimum sample size was calculated by G*Power 3.1 statistical tool [42,43]. $N \geq (50+8\ m)/f^2$ (where m = number of independent variables and $f^2 = R^2/(1\text{-}R^2) = 0.15$ (medium effect size), $\alpha = 0.05$ and power $(1\text{-}\beta) = 0.95$ using G*Power 3.1 statistical tool [42,43]. The independent variables include (i) age, (ii) gender (categorical), (iii) body mass index (BMI), (iv-v) CETP gene variants −629 and 277 variants (categorical variable), (vi) Total cholesterol, (vii) Triglycerides, (viii) LDL cholesterol, (ix) Apolipoprotein A1 and (x) Apolipoprotein B. For m = 10, n = 178. However, for population representative data more than 300 sample is recommended for multiple regression analysis [44]. In this study, n = 402 sample was included.

2.3. Specimen collection and storage

For genetic and biochemical analyses, written informed consent was obtained from all the participants. This study was performed in line with the principles of the Declaration of Helsinki. The Ethical Review Committee of the Department of Biochemistry and Molecular Biology, approved the study at the University of Dhaka. Additionally, the samples and data were treated anonymously. Five millilitres (5 mL) of blood samples were collected in the morning following 10–12 h of fasting and fed a normal diet during the last 3 days, maintaining all aseptic precautions, and whole blood and serum samples were stored at −80 °C.

2.4. Extraction of genomic DNA

Genomic DNA was extracted using the procedure as described previously [45–47]. Briefly, DNA was extracted from peripheral blood leukocytes via the commercially available FavorPrep Blood Genomic DNA Extraction Kit (Favorgen, Biotech Corp, Taiwan), which uses a silica-based separation technique optimized in the laboratory. Firstly, 200 μl of whole blood sample was transferred to a microcentrifuge tube and 20 μl PureLink™ RNase A (20 mg/mL) (Thermo Fisher Scientific Inc., USA) was added and incubated for 2 min at room temperature. Then lysed the sample with FABG Buffer (200 μl) and Proteinase K (20 μl) incubating at 60 °C for 15 min. Then after a brief spinning, 200 μl molecular-grade ethanol (96–100 %) was added to the sample and mixed thoroughly by pulse-vortexing for 10 s. The mixture was then transferred to a FABG Mini Column. After centrifuging at 6,000×*g* for 1 min, the column was washed with 400 μl of W1 Buffer and 750 μl Wash Buffer, discarding the flow-through each time by centrifuging at 18, 000×*g* for 30 s. Finally, the DNA was eluted by adding preheated Elution Buffer (100 μl) to the column, incubating briefly, and centrifuging at full speed to collect the purified DNA in a new tube. The DNA was stored at −27 °C for long-term use.

2.5. PCR amplification of target regions

After the DNA concentration was measured in a Nanodrop spectrophotometer, 5–10 ng was used for standard polymer chain reaction (PCR) of the CETP gene via 2 sets of primers in a 10 μl reaction volume. A 127 bp region of the CETP −629 locus was amplified via 0.4 μl of 10 pmol/μl forward and 0.4 μl of 10 pmol/μl reverse primers (5′- AGA ATT GAA ATG CCA CAG ACA TTC C-3′ and 5′- CCT TGA TAT GCA TAA AAT AAC TCT CG-3′, respectively) (Macrogen, Inc., Korea). The reaction mixture also contained 0.2 μl (10 mM) dNTPs (New England Biolabs, USA), 0.05 μl (5000 U/mL) one Taq quick-load DNA polymerase (New England Biolabs, USA), 2.0 μl one Taq quick-load reaction buffer (New England Biolabs, USA), 3 μl of extracted DNA and 3.95 μl nuclease free water.

The 535 bp region containing the 277 locus was amplified in a 10 μl reaction volume using 0.3 μl (10 pmol/μl) forward and 0.3 μl (10 pmol/μl) reverse primers (5′- CAC TAG CCC AGA GAG AGG AGT GCC-3′ and 5′- CTG AGC CCA GCC GCA CAC TAA C-3′, respectively) (Macrogen Inc., Korea), 0.05 μl (5000 U/mL) one Taq quick-load DNA polymerase (New England Biolabs, USA), 0.2 μl (10 mM) dNTPs (New England Biolabs, USA), 2.0 μl one Taq quick-load reaction buffer (New England Biolabs, USA), 3.0 μl DNA extract, 0.5 μl dimethyl-sulfoxide (Wako, Japan) and 3.65 μl nuclease free water.

The thermal cycling conditions were as follows: initial denaturation at 94 °C (3 min) for every SNP; denaturation at 94 °C and 95 °C (30 s); annealing at 58 °C and 62 °C (30 s); extension at 68 °C (30 s) and 72 °C (45 s); and a final extension at 68 °C (5 min) and 72 °C (10 min) for the −629 and 277 SNPs, respectively. The denaturation and extension steps were repeated 35 times.

2.6. Genotyping by RFLP

After confirmation of the PCR amplicon via agarose gel electrophoresis, 5 μl of the −629C/A- and 277C/T-containing PCR products were digested at 37 °C for 30 min in a 10 μl reaction mixture containing 0.4 μl (10 U/L) AvaI (New England Biolabs, USA) and 0.3 μl (20 U/L) TaqI-v2 (New England Biolabs, USA) restriction enzymes, respectively. For the digestion of 127 bp PCR products of the CETP −629 locus, 1 μL of 10× buffer (rCutSmart™, New England Biolabs, USA) and 3.6 μl molecular grade water was added to make the final volume of 10 μl. For the 535 bp digestion of 277C/T, 1 μL of 10× buffer (rCutSmart™, New England Biolabs, USA) and 3.7 μl molecular grade water was added to make the final volume of 10 μl. The digestion was followed by agarose (3 %) gel electrophoresis and visualization of the DNA fragments. For better imaging and separation, we solidified the agarose gel for at least 1 h, as done in our previous studies [48,49]. The digested product was visualized using a gel documentation system following ethidium bromide staining. Picture was taken using a UV transilluminator.

Digestion by AvaI produced the following genotypes for SNP -629C/A with corresponding band sizes: 127 bp for −629AA, 127 and 100 bp for −629CA, and 100 bp for −629CC. Similarly, digestion by TaqI produced the following genotypes for SNP 277C/T: 535 bp for 277 TT; 535, 361, and 174 bp for 277CT; and 361 and 174 bp for 277CC.

2.7. Biochemical analysis

Plasma glucose concentrations were estimated via hexokinase (reference method) and total serum cholesterol, and triglycerides were measured via an end-point method via a Dimension® *RxL Max* automated chemistry analyzer (Siemens Healthineers, USA). In brief, glucose estimation employed the hexokinase (HK) that phosphorylates glucose present in the sample in presence of adenosine-5′-triphosphate (ATP) and magnesium to form glucose-6-phosphate (G-6-P) and adenosinediphosphate (ADP). G-6-P is then oxidized by glucose-6-phosphate dehydrogenase (G-6-PDH) in the presence of nicotinamide adenine dinucleotide (NAD) to produce 6-phosphogluconate and NADH. One mole of NAD is reduced to 1 mol of NADH for each mole of glucose present. The absorbance of the now formed NADH (corresponds the glucose concentration) is determined using a bichromatic (340 and 383 nm) endpoint technique. Dimension® clinical chemistry system automatically recognizes the flex reagent when inserted and prepare reagent as required. Before assay, the method was calibrated using a 3 point calibration procedure according to the recommendation of the manufacturer using calibrator (Siemens Healthineers. USA). After calibration, plasma samples were placed in the sample tray sequentially and programmed for glucose in a batch mode. The Dimension® system automatically added 3 μL of plasma into a freshly automatically prepared reaction cell in which 56 μL of reagent 1 and 321 μL of reagent 2 (water as diluent) were added. After incubation (37 °C) for 4 min, optical density was measured bichromatically at 340 and 383 nm and the system automatically calculated and printed the results. For quality control, Bio-rad quality control material level 1 and level 2 were used. The total cholesterol estimation principle used Cholesterol esterase (CE) that catalyzes the hydrolysis of cholesterol esters to produce free cholesterol which, along with preexisting free cholesterol, is oxidized to cholest-4-ene-3-one and hydrogen peroxide by cholesterol oxidase (CO). In the presence of horseradish peroxidase (HPO), the hydrogen peroxide thus formed is used to oxidize N,N diethylaniline-HCl/4-aminoantipyrine (DEA-HCl/AAP) to produce a chromophore that absorbs light at 540 nm. A polychromatic end point absorbance technique (452, 540, 700 nm) is used measure the oxidized DEA-HCl/AAP which is directly proportional to the total cholesterol concentration in the sample. After calibration, serum samples were placed in the sample tray sequentially and programmed for total cholesterol, triglyceride. The Dimension® system automatically added 3 μL of serum into a freshly automatically prepared reaction cell in which 88 μL of reagent 1 and 26 μL of reagent 2 were added. After incubation (37 °C), optical density was measured and the system automatically calculated and printed the results. The triglycerides estimation method is based on the enzymatic procedure that the enzyme lipoprotein lipase (LPL) to generate free glycerol from triglycerides. Glycerol kinase (GK) phosphorylates glycerol by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate which upon oxidation by Glycerol-3-phosphate-oxidase produces dihydroxyacetone phosphate and H_2O_2. The now produced H_2O_2 is consumed by the 4-aminoantipyrine by peroxidase (POD) with the formation of chromogenic quinoneimine (λ_{max} 510) can be measured using a bichromatic (510, 700 nm) end-point technique and is directly proportional to the concentration of triglycerides present in the sample. Before assay, the method was calibrated using a 3 point calibration procedure according to the recommendation of the manufacturer using CHEM II calibrator (Siemens Healthineers, USA). After calibration, serum samples were placed in the sample tray sequentially and programmed for triglyceride in a batch mode. The Dimension® system automatically added 4 μL serum into a freshly automatically prepared reaction cell in which 133 μL of reagent was added. After incubation (37 °C), optical density was measured.

Serum HDL-c was measured via a detergent-based direct homogeneous assay via a Beckman Coulter AU480 chemistry analyzer (Beckman Coulter Inc., USA). The HDL-c quantification employs a two phase homogenous reagents for the selective measurement of serum HDL-c in the presence of other lipoprotein particles. In the first phase, 2 μl of serum was transferred to the reaction cell in which 200 μl of the first reagent was added automatically by the analyzer that solubilized the free cholesterol in LDL, VLDL and Chylomicrons and converted to a colorless end product by cholesterol oxidase, peroxidase, and N,N-bis(sulfobutyl)-m-toluidine-disodium (DSBmT). In the second phase, 66 μl of the second reagent was added. The unique detergent then selectively solubilizes HDL-lipoproteins to release cholesterol from HDL particles which upon reaction with cholesterol esterase, cholesterol oxidase generates Cholest-4-en-3-one and H_2O_2. The H_2O_2 so formed reacts with the chromogen 4-aminoantipyrine and yield a blue color complex which was measured bichromatically at 600/700 nm. Serum LDL cholesterol was calculated from total cholesterol subtracting HDL cholesterol and very-low-density lipoprotein (VLDL) cholesterol [Friedewald Formula] [50]. Serum Apolipoprotein A1 (ApoA1) was measured by Immunoturbidimetric end-point method using a Beckman Coulter AU480 chemistry analyzer (Beckman Coulter Inc., USA). The system uses 52 μl Reagent 1 (buffer) and 52 μl of Reagent 2 (human ApoA1-antibody) in 1.6 μl of serum to yield insoluble aggregates that was measured at 540 nm after 8.5 min of incubation at 37 °C. The absorbance of these aggregates is proportional to the Apo A1 concentration in the sample. Similarly, Apolipoprotein B (ApoB) was measured using the kit that reacts specifically with ApoB in the serum (1.6 μl) when mixed with 52 μl of Reagent 1 (buffer) and 28 μl of Reagent 2 to form aggregates that was measured at 340 nm (Beckman Coulter Inc., USA).

2.8. Classification of dyslipidemia

For the classification of dyslipidemia, the NCEP-ATPIII guidelines were followed [51]. In brief, total cholesterol (TC) was classified as elevated when TC values $\geq$ 200 mg/dl, elevated triglycerides $\geq$150 mg/dl), low HDL-c (female) $<$ 50 mg/dl, (male) $<$ 40 mg/dl and elevated LDL-c $\geq$130 mg/dl.

2.9. Statistical analysis

The distribution of the HDL-c data was tested for normal distribution using the Kolmogorov-Smirnov test with MedCalc® version 11.4 (www.medcalc.org). Since the HDL-c data followed a normal distribution ($P = 0.085$), the data were presented as mean ± SD or number (%). To check whether the genotypes followed the Hardy-Weinberg equilibrium (HWE), Chi-squared and p values were determined using the CHIDIST function of Microsoft Excel 2007 and cross-checked for Chi-squared

values using online tools (https://wpcalc.com/en/equilibrium-hardy-weinberg/). To determine the difference in genotype distribution and allele frequency of the CETP gene variants (at −629 and 277 loci) between the high HDL-c and low HDL-c groups, Fisher's exact test was used. This test compared the number of mutant heterozygous, mutant homozygous genotypes, and allele frequency between the low HDL-c and high HDL-c groups, considering the number of wild homozygous as a reference with OR (95 % CI) and p values using GraphPad Prism 5.04 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com) in co-dominant, dominant and recessive models to find out the impacts of gene variants on HDL-c. HDL-c and other lipid parameters as well as ApoA1 and ApoB was also compared between genotypes (at both loci) of the CETP gene variants by independent sample *t*-test to determine the variation in the lipids and lipoproteins levels with the variation at gene loci. To find out the association of the CETP gene variants, multiple linear regression analyses were performed considering HDL-c as dependent variable and gene variants for CETP −629 and 277 genotypes as independent variables with adjustment of the relevant confounders (age, gender, body mass index, total cholesterol, Triglycerides, LDL cholesterol, Apolipoprotein A and Apolipoprotein B using MedCalc® version 11.4 for Windows (MedCalc Software, www.medcalc.org).

2.10. Evaluation of the regulatory role of the CETP −629C/A and 277C/T polymorphisms

From the Ensembl database, we curated the genomic information of these 2 SNPs, where we found that CETP −629C/A is an intergenic SNP and that the other SNP is an intronic SNP. Therefore, we tried to discern whether these genes play regulatory roles. For this purpose, we used RegulomeDB, and then, to assess the tissue specificity of CETP gene expression, we explored the Human Protein Atlas.

3. Results

3.1. Characteristics of the study subjects

In this study, a total of 402 subjects were included according to the inclusion-exclusion criteria; among them, 54 % were male, and the remaining 46 % were female (Table 1). The mean ± SD (range) age was 38.4 ± 8.0 (20–65) years. The systolic (SBP) and diastolic (DBP) blood pressures were 112 ± 12 and 76 ± 7 mmHg, respectively. The fasting blood glucose (FBG), creatinine and alanine aminotransferase (ALT) levels were 4.9 ± 0.6 mmol/L, 0.88 ± 0.20 mg/dL and 30 ± 9 U/L, respectively.

3.2. Lipid profile of the study participants

The lipidemic status of the study subjects is shown in Table 2 and Table 3. Reference range for high HDL-c is: ≥40 mg/dl for male and ≥50 mg/dl for female. Reference range for low HDL-c is < 40 mg/dl for male and <50 mg/dl for female. The mean ± SD of HDL-c of the total subjects was 34.9 ± 7.9; 32.4 ± 6.8 in males and 37.8 ± 8.0 females. Among the total sample, 196 (90.3 %) males had HDL-c levels <40 mg/dL, 174 (94.1 %) females had HDL-c levels <50 mg/dL, and 370 (92.0 %) subjects had HDL-c levels below the reference values. Overall, of the total 402 subjects, only n = 32 subjects had high HDL-c (≥40 mg/dl for male and ≥50 mg/dl for female) and the majority (n = 370) had low HDL-c [below the reference range (<40 mg/dl for male and <50 mg/dl for female)].

Table 1
Characteristics of the study subjects ($n = 402$).

Variables	Mean ± SD	Number (%)
Age (years)	38.4 ± 8.0	–
Gender (Male/Female)	–	217 (54.0 %)/185(46.0 %)
BMI (kg/m^2)	24.7 ± 4.1	–
SBP (mmHg)	112 ± 12	–
DBP (mmHg)	76 ± 7	–
FPG (mmol/L)	4.9 ± 0.6	–
Creatinine (mg/dL)	0.88 ± 0.20	–
ALT (U/L)	30 ± 9	–

Results are expressed as mean ± SD; BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; FPG, Fasting plasma glucose; ALT, Alanine aminotransferase.

Table 2
Lipidemic status of the study subjects ($n = 402$).

Variables	Mean ± SD
Total cholesterol (mg/dL)	173 ± 38
HDL cholesterol (mg/dL)	34.9 ± 7.9
In Male, HDL cholesterol (mg/dL)	32.4 ± 6.8
In Female, HDL cholesterol (mg/dL)	37.8 ± 8.0
LDL cholesterol (mg/dL)	109 ± 34
Triglycerides (mg/dL)	144 ± 88

Results are expressed as mean ± SD; HDL, High-density lipoprotein; LDL, Low-density lipoprotein.

Table 3
Proportion of dyslipidemia among the study subjects ($n = 402$).

Variables	Number n (%)
Elevated total cholesterol (≥200 mg/dl)	84 (20.9 %)
Low HDL cholesterol (<40 mg/dl) for male, (<50 mg/dl for female)	370 (92.0 %)
In Male, Low HDL cholesterol (<40 mg/dl)	196 (90.3 %)
In Female, Low HDL cholesterol (<50 mg/dl)	174 (94.1 %)
Elevated LDL cholesterol (≥130 mg/dl)	91 (22.6 %)
Elevated triglycerides (≥150 mg/dl)	136 (33.8 %)

Results are expressed as number (%); Proportion of dyslipidemia was determined using the cut-off values indicated in parenthesis; HDL, High-density lipoprotein; LDL, Low-density lipoprotein.

3.3. Genotypic and allelic frequencies of CETP gene polymorphisms

CETP −629 C/A (rs1800775) genotype analysis was performed via the PCR-RFLP method (Fig. 1). In the case of the CETP −629 C/A variant, the genotypic frequencies of CC, CA, and AA were 12.2 %, 45.3 %, and 42.5 %, respectively (Table 4). This variant's allelic frequency for the C and A alleles was 0.35 and 0.65, respectively. CETP 277 C/T (rs708272) genotyping was also conducted via PCR-RFLP (Fig. 2). The genotypes of the CETP 277 C/T SNP are CC, CT, and TT, which were found to have frequencies of 25.1 %, 48.5 %, and 26.4 %, respectively (Table 4). Furthermore, the occurrence and frequency of C and T alleles were 0.49 and 0.51, respectively.

3.4. Hardy–Weinberg model assessment

The Hardy–Weinberg equilibrium was used to analyze the constancy of the genotype frequencies observed in this study. Analysis of Hardy–Weinberg equilibrium revealed that the genotypic and allelic frequencies of the CETP −629C/A and 277C/T polymorphisms remained constant in the studied population (n = 402) (Table 4). As the p-value is > 0.05, this implies that there was no deviation of genotypic and allelic frequencies from the Hardy–Weinberg principle in our studied samples.

3.5. Comparison of CETP −629C/A genotypes between HDL-c groups

To determine whether the −629C/A polymorphism and low HDL-c levels differed significantly, a genetic model (e.g. codominant, dominant and recessive models) analysis was conducted between the HDL-c groups. In the codominant model, both alleles contribute to the phenotype, meaning heterozygous individuals exhibit an intermediate

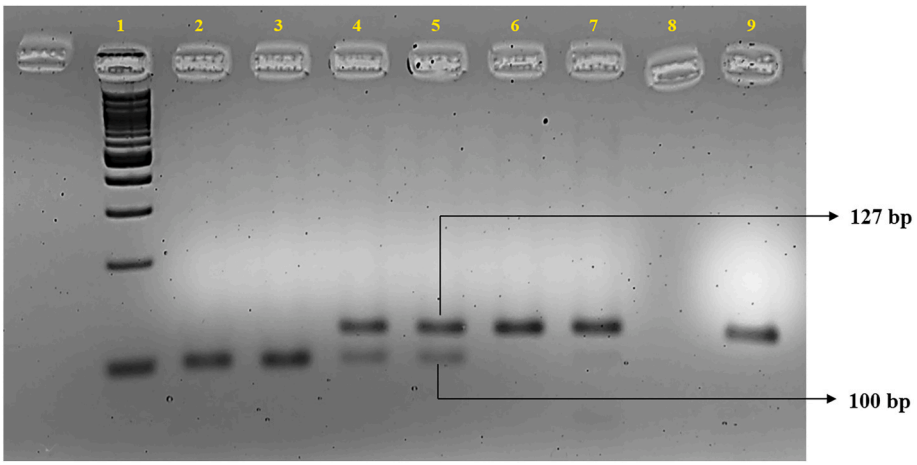

Fig. 1. Gel images of restriction digestion products of different genotypes of CETP −629C/A (rs1800775). The 100 bp fragment in lanes 2 and 3 indicates the CC genotype. The presence of 100 bp and 127 bp in lanes 4 and 5 indicates a heterozygous CA genotype, while the presence of 127 bp in lanes 6 and 7 indicates the AA genotype. Lane 1 contains a 100 bp DNA ladder.

Table 4
Genotypic and allelic frequencies of −629C/A and 277C/T polymorphisms of CETP gene and their Hardy Weinberg Equilibrium analysis ($n = 402$).

CETP Gene loci	Genotype Frequency				Allelic Frequency	
−629C/A	CC [n (%)]	CA [n (%)]	AA [n (%)]	*HWE p*-value	C	A
	49 (12.2 %)	182 (45.3 %)	171 (42.5 %)	0.957	280 (0.35)	524 (0.65)
277C/T (Taq1B)	CC [n (%)]	CT [n (%)]	TT [n (%)]		C	T
	101 (25.1 %)	195 (48.5 %)	106 (26.4 %)	0.552	397 (0.49)	407 (0.51)

HWE, Hardy Weinberg Equilibrium.

effect between the two homozygous groups. The dominant model assumes that the presence of a single copy of the variant allele is sufficient to influence the trait, so heterozygous and homozygous variant individuals are grouped together and compared against the reference homozygous group. In contrast, the recessive model considers the effect only when two copies of the variant allele are present, meaning homozygous variant individuals are compared against the combined group of heterozygous and reference homozygous individuals.

Table 5 shows that while the −629AA genotype was less common in the low-HDL-c group [41.6 % vs. 53.1 % (p = 0.576, OR = 0.59 (*0.17 to 2.11*)], the −629CC and CA genotypes were more common in the low-HDL-c group [(12.4 % vs. 9.4 %; 46.0 % vs. 37.5 %) (p = 1.000, OR = 0.92, 95 %CI = 0.25–3.41)] respectively in co-dominant model. Additionally, genotype distribution in the dominant and recessive models showed no significant difference between the low and high HDL-c groups (Table 5). There were no discernible variations in the frequencies of the −629C or A alleles between the groups with low and high HDL-c groups [0.35 vs. 0.28 for −629C and 0.65 vs. 0.72 for A (p = 0.275, OR = 0.71, (95 % CI = 0.41–1.23) respectively] (Table 5).

3.6. Evaluation of CETP 277 C/T genotypes between HDL-c groups

Genetic models (e.g. codominant, dominant and recessive models) were analyzed to compare 277 C/T genotypes between the 2 groups of HDL-c (Table 6). The CETP 277 C/A polymorphism genotype and allele frequency were significantly different between the HDL-c groups in the codominant and recessive models. Assessment of the TT genotype against the CC + CT genotypes in recessive model yielded a p value 0.011 [OR = 0.37, 95 % CI = 0.18–0.78] implies the protective role of

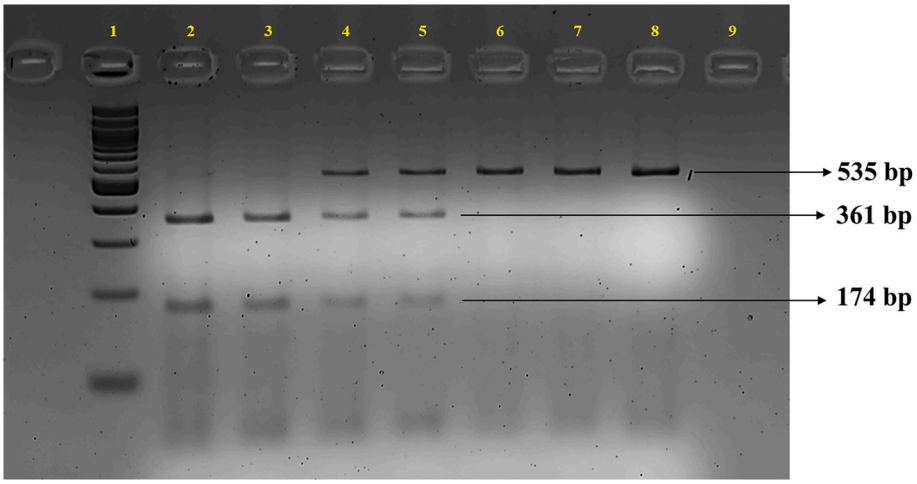

Fig. 2. Gel images of restriction digestion products of different genotypes of CETP 277 C/T (rs708272). The 361 bp and 174 bp fragments in lanes 2 and 3 indicates the CC genotype. The presence of 535 bp, 361 bp and 174 bp in lanes 4 and 5 indicates a heterozygous CT genotype, while the presence of 535 bp in lanes 6 and 7 indicates the TT genotype. Lane 1 contains a 100 bp DNA ladder.

Table 5
Comparison of CETP −629 C/A genotypes between high HDL-c and low HDL-c groups ($n = 402$).

Genetic Model	Genotype	High HDL-c ($n = 32$)	Low HDL-c ($n = 370$)	OR (*95 % CI*)	*p*-value
Co-dominant	CC	3 (9.4 %)	46 (12.4 %)	Ref	–
	CA	12 (37.5 %)	170 (46.0 %)	0.92 *(0.25 to 3.41)*	1.000
	AA	17 (53.1 %)	154 (41.6 %)	0.59 *(0.17 to 2.11)*	0.576
Dominant	CC	3 (9.4 %)	46 (12.4 %)	Ref	–
	CA + AA	29 (90.6 %)	324 (87.6 %)	0.73 *(0.21 to 2.41)*	0.783
Recessive	CC + CA	15 (46.9 %)	216 (58.4 %)	Ref	–
	AA	17 (53.1 %)	154 (41.6 %)	0.63 *(0.31 to1.30)*	0.263
Alleles	*C*	*18 (0.28)*	*262(0.35)*	0.71 *(0.41to 1.23)*	0.275
	A	*46 (0.72)*	*478 (0.65)*		

HDL-c, High-density lipoprotein cholesterol; OR, Odds Ratio; CI, Confidence interval; The genotypes and allele frequency was compared between the High HDL-c and Low HDL-c groups by Fisher's exact test.

the TT genotype (T allele).

Comparison of HDL-c between genotypes are presented in Table 7. For the −629 locus, serum HDL-c was higher in AA ($p = 0.023$) compared to CC in co-dominant model, CA + AA ($p = 0.043$) compared to CC in dominant model (Table 7). For the 277 locus, serum HDL-c was higher in TT against CC ($p = 0.002$) in co-dominant model, in CT + TT against CC ($p = 0.019$) in dominant model and in TT against CC + CT ($p = 0.003$) recessive model (Table 7). With a p value of 0.051, the allelic frequencies of the C and T alleles of the CETP 277 C/T polymorphism differed marginally (Table 6). The comparison of other lipid parameters and lipid fractions between genotypes has been shown in Supplementary Table 1. Comparison of total cholesterol, Triglycerides, LDL cholesterol, ApoA1 and ApoB between genotypes at CETP −629 and 277 loci (Supplementary Table 1) showed no significant difference of the lipid and lipoproteins between genotypes.

3.7. Association of low HDL-c with the CETP −629C/A and 277C/T polymorphisms

TC, TG, LDL-c, ApoA1, and ApoB showed no significant genotype differences. Multiple linear regression, with HDL-c as the dependent variable and CETP variants and confounders as independent variables, revealed inverse associations for −629CC against CA + AA ($\beta = -1.106$, $p = 0.038$) and 277CC + CT against TT ($\beta = -0.963$, $p = 0.016$) with serum HDL-c (Table 8).

3.8. Evaluation of the regulatory role of the CETP −629C/A and 277C/T polymorphisms

RegulomeDB predicted the probability of the regulatory effect of these 2 SNPs to be 0.553 and 0.554, respectively, with both of their ranks being 1f (Table 9). Rank 1f implies that the likelihood of these 2 SNPs being functional in regulation is greater with enough supportive data on the eQTL/caQTL + TF binding/chromatin accessibility peak. After the ChIP data were analyzed, we observed that −629C/A intersected with the ChIP peaks or, in other words, binding sites of the PRDM1, AHDC1, NR2F2, ZNF221, POLR2A, ZBTB20, CEBPG, TFAP2B, and ZNF687 transcription factors. In addition, this SNP also falls within the DNase hypersensitivity site. The Human Protein Atlas revealed that the CETP gene is specifically expressed in the liver, adipose tissue, lymphoid tissue, and placenta. In the liver, the chromatin state of highly active enhancers was found to interact with this SNP, indicating its possible role in the gene expression of this CETP gene itself.

On the other hand, for the 277C/T polymorphism, the RegulomeDB revealed that the binding sites of the ESRRA, ZNF687, and CEBPA transcription factors cover this SNP location (Table 9). Moreover, transcription and active enhancer chromatin states interact with this SNP in the liver, where the CETP gene is expressed at the highest level [52]. These findings demonstrate that the 277C/T polymorphism might be involved in the regulatory role and gene expression of this CETP gene.

Table 6
Evaluation of CETP 277 C/T genotypes between HDL-c groups ($n = 402$).

Model	Genotype	High HDL-c ($n = 32$)	Low HDL-c ($n = 370$)	OR (*95 % CI*)	p-value
Co-dominant	CC	7 (21.9 %)	94 (25.4 %)	Ref	–
	CT	10 (31.2 %)	185 (50.0 %)	1.38 (0.51–3.74)	0.600
	TT	15 (46.9 %)	91 (24.6 %)	0.45 (0.18–1.16)	0.115
Dominant	CC	7 (21.9 %)	94 (25.4 %)	Ref	–
	CT + TT	25 (78.1 %)	276 (74.6 %)	0.82 *(0.34 to 1.96)*	0.832
Recessive	CC + CT	17 (53.1 %)	279 (75.4 %)	Ref	–
	TT	15 (46.9 %)	91 (24.6 %)	0.37 *(0.18 to 0.78)*	**0.011**
Alleles	*C*	*24(0.38)*	*373(0.50)*	0.59 *(0.35 to 1.00)*	0.051
	T	*40(0.62)*	*367(0.50)*		

HDL-c, High-density lipoprotein cholesterol; OR, Odds Ratio; CI, Confidence interval; The genotypes and allele frequency was compared between the High HDL-c and Low HDL-c groups by Fisher's exact test.

Table 7
Comparison of HDL-c between genotypes at CETP −629 and 277 loci.

Model	CETP −629			CETP 277		
	Genotype	HDL-c (mg/dl)	*P*-value	Genotype	HDL-c (mg/dl)	*P*-value
Co-dominant	CC ($n = 49$)	32.8 ± 5.4	Ref	CC ($n = 101$)	33.4 ± 7.3	Ref
	CA ($n = 182$)	34.7 ± 7.9	0.096	CT ($n = 195$)	34.6 ± 7.4	0.171
	AA ($n = 171$)	35.7 ± 8.4	**0.023**	TT ($n = 106$)	36.9 ± 8.9	**0.002**
Dominant	CC ($n = 49$)	32.8 ± 5.4	Ref	CC ($n = 101$)	33.4 ± 7.3	Ref
	CA + AA ($n = 353$)	35.2 ± 8.1	**0.043**	CT + TT ($n = 301$)	35.4 ± 8.0	**0.019**
Recessive	CC + CA ($n = 231$)	34.3 ± 7.4	Ref	CC + CT ($n = 296$)	34.2 ± 7.4	Ref
	AA ($n = 171$)	35.7 ± 8.4	0.101	TT ($n = 106$)	36.9 ± 8.9	**0.003**

HDL-c, High-density lipoprotein cholesterol; HDL-c values between genotypes were compared by independent sample *t*-test.

Table 8
Association of HDL-c with CETP −629C/A and 277C/T polymorphisms.

Independent variables	Coefficient β	t-value	p-value
Age (years)	0.005	0.221	0.825
Gender (F)	1.343	3.676	<0.001
BMI (kg/m^2)	−0.161	−3.660	<0.001
CETP −629 CC against (CA + AA)	−1.106	−2.081	0.038
CETP 277 CC + CT against TT	−0.963	−2.426	0.016
TC (mg/dl)	0.050	5.774	<0.001
TG (mg/dl)	−0.021	−8.955	<0.001
LDL-c (mg/dl)	−1.660	−2.583	0.010
ApoA1 (mg/dl)	0.252	32.117	<0.001
ApoB (mg/dl)	−0.079	−7.228	<0.001

BMI, Body mass index; CETP, Cholesteryl ester transfer protein; TC, Total cholesterol; TG, Triglycerides; LDL-c, Low-density lipoprotein cholesterol; ApoA1, Apolipoprotein A1; ApoB, Apolipoprotein B; Multiple linear regression analysis was done considering HDL cholesterol values as dependent variable and age, gender, BMI, CETP −629 and 277 gene variants, TC, TG, LDL-c, ApoA1 and ApoB values as independent variables.

Table 9
Evaluation of regulatory functions from RegulomeDB.

rs IDs	probability	ranking	Interacting TFs
rs1800775	0.55436	1f	PRDM1 AHDC1 NR2F2 ZNF221 POLR2A ZBTB20 CEBPG TFAP2B ZNF687
rs708272	0.55436	1f	ESRRA ZNF687 CEBPA

TFs = Transcription Factors.

4. Discussion

We studied lipids, LDL, and cholesterol acyltransferase in our earlier research [53–57], and this time we're looking at cholesteryl ester transfer protein. This study was carried out to examine the genetic variations of the study subjects' CETP gene (−629C/A and 277C/T (Taq1B)) in order to investigate the connection between CETP gene polymorphisms and circulating HDL cholesterol in people from Bangladesh. In this study, our population genetics analysis revealed that the −629 CC genotype is associated with lower serum HDL-c levels, indicating a negative effect, whereas the presence of the A allele (CA + AA genotypes) appears to have a protective role. Thus, the C/A transversion at the −629 locus were associated with higher HDL-c.

Our findings is consistent with most of the studies conducted on other populations that reported the positive contribution of the −629A allele to circulating HDL-c [30,33–35,37], but inconstant with the findings of a multiethnic Singaporean community [32]. Compared with the C allele, the CETP −629A allele increases HDL-c in control subjects of European ancestry [34]. Similarly, another population-based study conducted on the Iranian population reported higher HDL-c in the −629A allele than in the C allele [33]. Wang et al. reported increased HDL-c in the −629A allele in the Chinese population [37]. A multiethnic study of atherosclerosis consisting of 6,814 subjects confirmed that the −629A allele is associated with a reduction in CETP activity and an increase in circulating HDL-c [35]. A meta-analysis also supported the above findings [30]. In this study, the −629C and A allele frequencies were 0.35 and 0.65, respectively, which are closer to those reported in South Asia (C = 0.3862, A = 0.6138) but differ from those reported in other populations. However, our result differed from findings of a multiethnic Singaporean community that includes Indian, Chinese, and Malay people [32]. These slight discrepancy in our findings may be due to differences in allelic variation, while environmental factors could contribute to variations in HDL-c levels.

Our study also revealed that the 277CC + CT is negatively associated with serum HDL-c and TT is protective (as presented in Table 6, 7 and 8). On the other hand, the 277CC + CT is more frequent and TT is less frequent in the low HDL-c group (Table 6), and TT showed higher levels of serum HDL-c (Table 7) compared to CC + CT. Multiple linear regression showed that the 277CC + CT is negatively associated with serum HDL-c whereas the 277 TT is significantly positively associated with higher serum HDL-c i.e., T allele is protective. These findings are also consistent with most of the studies conducted in different populations. This transition leads to a reduction in the binding affinity of the splicing factor and represses its expression, leading to lower circulating CETP activity and higher HDL-c [34]. The 277T and −629A alleles are associated with lower CETP activity and higher HDL-c [33]. In a study conducted on Argentine people, CETP 277 TT was found to have a positive effect on circulating HDL-c [58]. In people in Japan, higher HDL-c is observed in the 277 TT genotype [39]. A multiethnic study of atherosclerosis consisting of 6,814 subjects confirmed that the 277T and −629A alleles are associated with a reduction in CETP activity and an increase in circulating HDL-c [35]. It has also been shown that higher HDL-c is related to Chinese men in the multiethnic Singaporean population [32], but another study conducted in the Chinese population revealed no association between the 277C/T transition and HDL-c [37]. A positive association between the 277C/T polymorphism and serum HDL-c has been reported in people in Pakistan [31]. In people in Tamilnadu, India, only a gender-based positive association was observed in the 277C/T transition for males [38]. Although several studies have been conducted on the Asian population, few have been conducted in South Asia [38,41], and particularly among people in Bangladesh, where low HDL-c is higher in the general population [10]. Population-based studies conducted in different populations reported a strong relationship between the CETP 277C/T transition and serum HDL-c, and CETP plays a key role in controlling circulating HDL-c and differences in allele frequency between Indian subjects with CAD [59] and other populations [35,60]. Moreover, the CETP 277C and T allele frequencies were 0.49 and 0.51, respectively, which are closer to those reported in South Asia (C = 0.5126, T = 0.4874) but differ from those reported in other populations.

In this study, we also evaluated the CETP −629C/A and 277C/T loci in RegulomeDB and reported that these 2 SNPs are the binding sites of transcription factors and possibly play important roles in their expression. Importantly, the in-silico analysis revealed that both CETP −629C/A (rs1800775) and 277C/T (rs708272) polymorphisms have a high likelihood of regulatory functionality, with RegulomeDB rankings of 1f and probabilities of 0.553 and 0.554, respectively. The −629C/A SNP was found to intersect with multiple transcription factor binding sites, including PRDM1, AHDC1, NR2F2, and POLR2A, and was located within a DNase hypersensitivity site, indicating its potential role in chromatin accessibility and gene regulation. Furthermore, active enhancer chromatin states in the liver, where CETP is highly expressed, were associated with this SNP, suggesting its involvement in CETP gene expression. Similarly, the 277C/T polymorphism interacted with key transcription factors such as ESRRA, ZNF687, and CEBPA, and showed transcriptional and enhancer activity in the liver. These findings strongly support the potential regulatory roles of both SNPs in modulating CETP gene expression, which may have functional implications in lipid metabolism and cardiovascular risk.

On the basis of the results generated from this study, a low level of circulating HDL-c is the predominant lipid disorder in our population, and the 277CC, 277CT and −629CC genotypes of the CETP gene are significantly associated with low levels of HDL cholesterol in our population whereas −629C/A (i.e., CA and AA and 277 C/T (TT)) has been found to be protective (Table 6, Table 7). These findings may contribute to further regional research on HDL-c levels and CVD in Bangladesh.

5. Conclusion

The CETP −629 and 277 heterozygous genotypes predominated over the wild-type or mutant homozygous genotypes, and the allelic frequencies were comparable to those of South Asian populations. This study identified that the CETP −629C/A and 277C/T polymorphisms are associated with low HDL-c. Consequently, this may present a potential risk for the development and sustenance of cardiovascular diseases.

CRediT authorship contribution statement

Muhammad Saiedullah: Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Nurun Nahar Nila:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Zimam Mahmud:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis. **Sonia Tamanna:** Writing – review & editing, Validation, Methodology, Formal analysis. **Md. Zahid Hassan:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Md. Zakir Hossain Howlader:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Ethics approval and consent to participate

The Ethical Review Committee of the Department of Biochemistry and Molecular Biology, approved the study at the University of Dhaka. Informed written consent was taken from all the study subjects before collecting samples.

Consent for publication

Not Applicable.

Funding

The study was partially funded by the Bangladesh Medical Research Council (BMRC), Bangladesh - FY 2018–2019.

Declaration of competing interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgements

We thank Shohanur Rahaman for his technical assistance with the PCR-RFLP assay.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2025.101992.

Data availability

All data generated or analyzed during this study are included in this manuscript.

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