c.*84G>A Mutation in CETP Is Associated with Coronary Artery Disease in South Indians

Mala Ganesan1*, Sheikh Nizamuddin1*, Shiva Krishna Katkam1, Konda Kumaraswami2, Uday Kumar Hosad3, Limmy Loret Lobo3, Vijay Kumar Kutala2, Kumarasamy Thangaraj1*

1 CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India, 2 Department of Clinical Pharmacology and Therapeutics, Nizam’s Institute of Medical Sciences (NIMS), Hyderabad, India, 3 Yashoda Super Specialty Hospital, Hyderabad, India

*These authors contributed equally to this work.
*thanga@ccmb.res.in

Abstract

Background
Coronary artery disease (CAD) is one of the leading causes of mortality worldwide. It is a multi-factorial disease and several studies have demonstrated that the genetic factors play a major role in CAD. Although variations in cholesteryl ester transfer protein (CETP) gene are reported to be associated with CAD, this gene has not been studied in South Indian populations. Hence we evaluated the CETP gene variations in CAD patients of South Indian origin.

Methods
We sequenced all the exons, exon-intron boundaries and UTRs of CETP in 323 CAD patients along with 300 ethnically and age matched controls. Variations observed in CETP were subjected to various statistical analyses.

Results and Discussion
Our analysis revealed a total of 13 variations. Of these, one 3'UTR variant rs1801706 (c.*84G>A) was significantly associated with CAD (genotype association test: OR = 2.16, 95% CI: 1.50–3.10, p = 1.88x10^-5 and allelic association test: OR = 1.92, 95% CI: 1.40–2.63, p = 2.57x10^-5). Mutant allele “A” was observed to influence the higher concentration of mRNA (p = 7.09x10^-3, R^2 = 0.029 and β = 0.2163). Since expression of CETP has been shown to be positively correlated with the risk of CAD, higher frequency of “A” allele (patients: 22.69% vs controls: 13%) reveals that c.*84G>A is a risk factor for CAD in South Indians.

Conclusions
This is the first report of the CETP gene among South Indians CAD patients. Our results suggest that rs1801706 (c.*84G>A) is a risk factor for CAD in South Indian population.
Introduction

Coronary artery disease (CAD) is the leading cause of mortality worldwide [1]. CAD and its clinical manifestations are etiologically complex, with approximately equal contributions from genetic and environmental factors [2,3]. Genetic risk scores derived from several functionally relevant single nucleotide polymorphisms (SNPs) or haplotypes in several genes may help in predicting CAD [4]. Associations of only a few common SNPs with CAD have been consistently replicated in several studies [5]. Both genome-wide association studies and candidate-gene approaches have identified a number of novel chromosome loci or genes that are associated with CAD [6,7]. However, they account for relatively small portion of the overall CAD risk; therefore, there is a need for identification of novel loci or genes for CAD. Cholesteryl ester transfer protein (CETP) gene is localized on chromosome 16, which is of 21,995 base pairs (bp) in size and consists of 16 exons (Gene ID 1071). CETP is a hydrophobic glycoprotein which plays a major role in RCT (Reverse Cholesterol Transport) from tissues to the liver. By enabling transfer of cholesteryl esters from high-density lipoproteins (HDL) to low density lipoproteins (LDL) and very low density lipoproteins (VLDL) lipoproteins CETP enables remodeling of plasma lipoproteins [8]. The elevated level of HDL cholesterol (HDL-C) is shown to be a protective factor for coronary artery disease from many epidemiological studies [9]. Our earlier studies have suggest that Indian populations are unique in their origin and practicing endogamy for the past thousands of years, hence expected to have unique set of mutation which led to several disease, cardiac disease in particular [10–12].

Among the fastest growing non-communicable diseases, cardiovascular diseases (CVDs) are expected to cause largest number of mortality and morbidity within India [13]. Indians possess a unique lipid profile characterized by high triglycerides, low high-density lipoprotein (HDL), and increased lipoprotein (a) levels [14]. CETP plays a major role in HDL metabolism and this gene possesses several SNPs that have been reported to be associated with plasma HDL concentrations. However, this gene has not been analysed on Indian population, hence this study was aimed to investigate whether CETP gene variations influence CAD in South Indian population.

Materials and Methods

2.1. Sample details

The study subjects composed of 323 CAD patients with coronary atherosclerosis and 300 age and ethnically matched healthy controls from South India. Blood samples were collected from Yashoda Hospital and Nizam Institute of Medical Sciences (NIMS), Hyderabad, India. Clinical manifestation of coronary atherosclerosis was evaluated by precutaneous coronary angiography, by a panel of experienced cardiologists. All ethnically matched control individuals were free from CAD, as determined by medical history, clinical examinations, or electrocardiography. Among CAD patients, 70% were males and 30% were females, while among the healthy control group 76% were male and 24% were female. The mean age of healthy controls was 65.26±10.30 years while that of patients was 56.21±10.45 years. In CAD patients; 18.57% were tobacco chewers, while it was 10% among the healthy controls. The demographic details of the individuals included in the study are given in Table 1.

We also utilized the genotype data of rs1801706/c.*84G>A of 1000 genome project’s samples from Ensembl genome browser (version 84) (asia.ensembl.org).
2.2. Sample collection and DNA isolation

Prior to collection of blood samples, CAD patients were subjected to physical/clinical examinations such as 12 lead ECG and lipid profile, etc. Blood samples of healthy controls from the same ethnic background, without hypertension or CAD based on electrocardiograph were collected. A total of 10 ml intravenous blood samples of both cases and controls were collected in EDTA vaccutainer, after obtaining informed written consent. Genomic DNA was isolated from all the samples using standard protocol [15]. This study followed the principles outlined in the Declaration of Helsinki (WMA World Medical Association Declaration of Helsinki), and was approved by the Institutional Ethics Committee of Yashoda Hospital, Hyderabad; Nizam’s Institute of Medical Sciences (NIMS), Hyderabad; and CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India.

2.3. Genotyping

The reference genomic sequence of CETP (ENSG00000087237) was obtained from the Ensemble database (asia.ensembl.org). Primers to amplify all the exons, and exon-intron boundaries of CETP were designed using Primer3 web version 4.0 (Table 2). PCRs (polymerase chain reactions) were performed using GeneAmp 9700 (Applied Biosystems, Foster City, USA) using Emerald Amp GT PCR master mix (TaKaRa) according to the manufacturer’s protocol. After PCR, Amplicons were size fractionated using 2% agarose gel, stained with ethidium bromide and observed under UV transilluminator. Subsequently, amplicons were treated with Exo-SAP (USB Corp., USA), and sequenced using a BigDye Terminator (v3.1) cycle sequencing kit (Applied Biosystems, Foster City, USA) on an ABI 3730XL DNA analyzer. Sequences obtained were assembled with the reference sequences using AutoAssembler software (Applied Biosystems, Foster City, USA). Variations observed were noted for further analysis.

2.4. Statistical analysis and functional validation

Allele and genotype frequencies were calculated by the allele counting method. Statistical comparisons were carried out by Plink software [16]. The P values less than 0.05 were considered for statistical significance. To explore the Hardy-Weinberg equilibrium (HWE), we consider...
genotype distribution in control samples and only those variants having HWE p value > 0.05 were utilized in further association analysis.

Further, to explore the functional significant of mutant allele “A” of rs1801706 (c.*84G>A), we utilized the genome expression dataset GSE6536 of HapMap population from GEO (gene expression omnibus) database [17] and genotype data of CETP with ±10 kb flanking region from ftp://ftp.ncbi.nlm.nih.gov/hapmap/genotypes/2009-01_phaseIII/plink_format/. Further, we extracted the population-wise normalized expression value of CETP specific probe GI_4557442 from above downloaded GSE6536 dataset and performed quantitative trait association analysis using Plink software [16]. To explore the group-wise differences of mRNA level, we performed t-test using R. Moreover, to conclude the relation between higher mRNA concentrations with CAD patients, we utilized the relationship discussed in previous reports. On the basis of Barkowski, RS et al. and Tan, MH [18,19];

\[
Concentration_{\text{HDL-C}} \propto \frac{1}{Concentration_{\text{mRNA-CETP}}}
\]

\[
Risk_{\text{CAD}} \propto \frac{1}{Concentration_{\text{HDL-C}}}
\]  

Hence, the genetic risk of CAD will be directly proportional to the mRNA concentration of CETP;

\[
Risk_{\text{CAD}} \propto Concentration_{\text{mRNA-CETP}}
\]

**Results**

We have investigated the exons, exon-intron boundaries and UTR of CETP in 323 individuals with CAD and 300 ethnically matched controls. In total, we found thirteen variants (SNPs), of which one was in splice regions [rs1532625 (C/T)]; eight were in introns [rs17231534 (C/A/T) and rs3816117 (T/C)] rs711752 (G/A), rs9930761 (T/C), rs11076176 (T/G), rs289714 (G/A), rs1800774(C/T) and rs289741(G/A)]; one was synonymous [rs5883(C/T)]; one was missense [rs1800777 (G/A)]; one was in 3' UTR [rs1801706 (G/A)]; and one was in the downstream position of gene [rs289743 (G/C)] (Table 3 and Fig 1).
Table 3. Genotype and allele frequency distributions of SNPs in CETP among cases and controls.

| SNP              | Gt/A* | CAD(N%/n=323) | Control(N%/n=300) | OR (95% CI) | P-value | HWE P-value |
|------------------|-------|---------------|-------------------|-------------|---------|-------------|
| rs17231534(C/A)  | CC    | 263(81.42)    | 260(86.66)        | 1.48(0.94–2.35) | 0.095   | −0          |
| Intron variant   | CA    | 12(3.71)      | 17(5.66)          |             |         |             |
|                  | AA    | 48(14.86)     | 23(7.66)          |             |         |             |
|                  | C     | 574(88.85)    | 537(89.5)         | 1.07(0.74–1.55) | 0.783   |             |
|                  | A     | 72(11.14)     | 63(10.5)          |             |         |             |
| rs3816117(T/C)   | TT    | 115(35.60)    | 150(50)           | 1.81(1.30–2.53) | 3.85E-04 | −0          |
| Intron variant   | TC    | 98(30.34)     | 24(8)             |             |         |             |
|                  | CC    | 110(34.05)    | 126(42)           |             |         |             |
|                  | T     | 328(50.77)    | 324(54)           | 1.2(0.94–1.52) | 0.146   |             |
|                  | C     | 318(49.22)    | 276(46)           |             |         |             |
| rs711752(G/A)    | GG    | 169(52.32)    | 152(50.66)        | 0.731(0.67–1.30) | 0.731   | −0          |
| Intron variant   | GA    | 89(27.55)     | 84(28)            |             |         |             |
|                  | AA    | 65(20.12)     | 64(21.33)         |             |         |             |
|                  | G     | 427(66.09)    | 388(64.66)        | 0.94(0.74–1.19) | 0.0637  |             |
|                  | A     | 219(33.90)    | 212(35.33)        |             |         |             |
| rs1532625(C/T)   | CC    | 99(30.65)     | 81(27)            | 0.84(0.58–1.20) | 0.36    | 0.422       |
| Splice region variant | CT  | 128(39.62)   | 143(47.66)        |             |         |             |
|                  | TT    | 96(29.72)     | 76(25.33)         |             |         |             |
|                  | C     | 326(50.46)    | 305(50.83)        | 0.84(0.58–1.20) | 0.36    |             |
|                  | T     | 320(49.53)    | 295(49.16)        |             |         |             |
| rs9930761 (T/C)  | TT    | 300(92.87)    | 282(94)           | 1.2(0.61–2.38) | 0.688   | −0          |
| Intron variant   | TC    | 22(6.81)      | 14(4.66)          |             |         |             |
|                  | CC    | 1(0.30)       | 4(1.33)           |             |         |             |
|                  | T     | 622(96.28)    | 578(96.33)        | 1.01(0.54–1.90) | 1       |             |
|                  | C     | 628(97.21)    | 587(97.83)        | 1.29(0.60–2.82) | 0.603   |             |
| rs5883(C/T)      | CC    | 305(94.42)    | 287(95.66)        | 1.3(0.59–2.88) | 0.599   | 0.701       |
| Synonymous variant | CT  | 18(5.57)     | 13(4.33)          |             |         |             |
|                  | TT    | 0(0)          | 0(0)              |             |         |             |
|                  | C     | 628(97.21)    | 587(97.83)        | 1.29(0.60–2.82) | 0.603   |             |
|                  | T     | 18(2.78)      | 13(2.16)          |             |         |             |
| rs11076176(T/G)  | TT    | 212(65.63)    | 192(64)           | 0.93(0.66–1.31) | 0.732   | 1           |
| Intron variant   | TG    | 101(31.26)    | 96(32)            |             |         |             |
|                  | GG    | 10(3.09)      | 12(4)             |             |         |             |
|                  | T     | 525(81.26)    | 480(80)           | 0.92(0.69–1.23) | 0.621   |             |
|                  | G     | 121(18.73)    | 120(20)           |             |         |             |
| rs289714(G/A)    | GG    | 23(7.12)      | 12(4)             | 0.54(0.25–1.17) | 0.129   | 0.436       |
| Intron variant   | GA    | 98(30.34)     | 107(35.66)        |             |         |             |
|                  | AA    | 202(62.53)    | 181(60.33)        |             |         |             |
|                  | G     | 144(44.29)    | 131(41.83)        | 0.97(0.74–1.28) | 0.99    |             |
|                  | A     | 502(77.70)    | 469(78.16)        |             |         |             |
| rs1800774(C/T)   | CC    | 180(55.72)    | 163(54.33)        | 0.95(0.68–1.31) | 0.788   | 0.0018      |
| Intron variant   | CT    | 108(33.43)    | 101(33.66)        |             |         |             |
|                  | TT    | 35(10.83)     | 36(12)            |             |         |             |
|                  | C     | 468(72.44)    | 427(71.16)        | 0.87(0.69–1.10) | 0.254   |             |
|                  | T     | 178(27.55)    | 173(28.83)        |             |         |             |
| rs1800777 (G/A)  | GG    | 309(95.66)    | 295(98.33)        | 2.67(0.89–8.61) | 0.089   | −0          |
| Missense variant | GA    | 14(4.33)      | 3(1)              |             |         |             |

(Continued)
Among these 13 variants, 6 were in HWE equilibrium (p > 0.05) (Table 3). Of which, rs1801706 (c.*84G>A) was significantly associated with patients group. The 3’ UTR variant c.*84G>A (G/A) showed a genotype distribution (%) of 60.37 (GG), 34.67 (GA) and 4.95 (AA) among individuals with CAD; whereas in controls the frequencies were 76.66 (GG), 20.66 (GA) and 2.66 (AA). Association analysis with genotype showed significant association with CAD (OR = 2.16, 95% CI: 1.50–3.10, p = 1.88x10^{-5}). The allelic distribution showed 77.70% of ‘G’ and 22.29% of ‘A’ in cases and among the controls the G showed 87% and ‘A’ showed 13% with significant association of the ‘A’ allele with OR = 1.92, 95% CI; 1.40–2.63, p-value 2.57x10^{-5}. Both genotype and allele distribution of CETP SNPs among cases and controls are given in Table 3. Since, we did not observed LD differences between the cases and controls; we did not proceed for haplotype analysis.

### 3.1. Functional validation of rs1801706/c.*84G>A

To functionally validate the mutant allele “A” (c.*84G>A), we have utilized the whole genome gene-expression data of 210 HapMap samples [17, 20] and performed QTL analysis with

![Observed variations and its location in CETP gene mRNA](https://doi.org/10.1371/journal.pone.0164151.g001)

**Fig 1.** Observed variations and its location in CETP gene mRNA. ENST00000200676 was utilized to represent the physical location of variants.
genotype information of same individuals from HapMap project, with additive model (Table 4) and observed variant rs1801706 in association with CETP mRNA level with p-value 7.09×10^-3 (R^2 = 0.029 and β = 0.2163) (Table 4). The genotype and normalized mRNA intensity/expression value for c.*84G>A is given in S1 Table. Interestingly, both heterozygous (GA) and mutant homozygous (AA) genotype were found to influence the higher level of mRNA (Fig 2). We also explored pair-wise comparison of mRNA expression between genotypes and observed that mRNA expression level of genotype AA vs. AG (p-value = 0.3452) and genotype AA vs. GG (p-value = 0.2632) were not significant, while genotype AG vs. GG was significantly different (p-value = 0.0018).

3.2. Prevalence of rs1801706/c.*84G>A

Further, we explored the frequency spectrum of rs1801706 (c.*84G>A) in other world populations, including Indians. We utilized the genotype dataset from 1000 genome project. We observed that only 3 populations were having frequency of <0.1 for rs1801706-A variant; (1) 0.086 in CDX (Chinese Dai), (2) 0.078 in MXL (Mexicans) and (3) 0.029 in PEL (Peruvians) (Table 5).

Discussion

CAD is caused by multiple genetic and environmental factors [2]. CETP plays a central role in human lipoprotein metabolism, as it facilitates the removal of excess cholesterol from the body via LDL receptor-mediated uptake in the liver and excretion into the bile [21]. Our earlier study on the -629 promoter of CETP gene had shown a significant association between CAD patients and controls [22]. Considering the crucial role of CETP in lipid metabolism, we investigated the association of genetic variants of the CETP with risk of coronary artery disease in patients from South India.

In the present study, we observed a 3’ UTR variant, rs1801706 (c.*84G>A) is associated with the CAD in South Indians, however, we did not find association of a few previously reported SNPs. A genome-wide linkage analysis conducted on healthy American woman cohort had analyzed over 350,000 SNPs and found only SNPs flanking or in the CETP gene were associated with both HDL-C and risk of incident CAD [23]. Papp et al. observed that rs5883/rs9930761C was associated with increased HDL-C levels in males [24]. Although our study group had 76% males, we did not find any significant association with rs5883. Studies on C>T/In9 (rs289714) was earlier shown to be associated with undesirable changes in adiposity and HDL-C levels when exposed to excessive calorie consumption [25,26], however, we did not find significant association between cases and the controls. Studies on Caucasians and African Americans [27] showed association of CETP variations with myocardial infarction (MI). The rs1800777 (Arg 451Gln) is located within the lipid-binding region of CETP protein and possibly may result in the loss of positive charge, varying the binding efficiency of CETP to cholesterol esters. Lu et al. reported that rs1800777 was associated with lower plasma HDL cholesterol levels [28], whereas Moleres et al. reported that this SNP is strongly associated with adiposity indexes [29]. However, in the present study both the genotype and allele frequency of this variant did not show any association with CAD. Interestingly, we found rs1801706 (c.*84G>A) was significantly associated with CAD, which is in agreement with Whitehall II et al.

Since, associated variant rs1801706 (c.*84G>A) was observed in UTR region, we predicted that mutant allele “A” might be affecting mRNA expression of CETP. Here, we were not aware that mutant allele “A” increasing or decreasing the expression. But, using Eq 2, it can be further predicted that mutant allele “A” should increase the expression because risk of CAD increases.
Table 4. SNPs present within ±10kb of CETP and their respective association p-value with normalized mRNA expression level. Only rs1801706 (c.*84G>A) was significantly associated and highlighted in bold.

| Chr. | SNP     | Physical position (hg19) | No. of subjects | β     | S.E.  | R²   | T     | P value |
|------|---------|--------------------------|-----------------|-------|-------|------|-------|---------|
| 16   | rs247617| 5699071                  | 247             | -0.0726 | 0.0691 | 0.0045 | -1.0510 | 0.2943  |
| 16   | rs6499863| 56992016                | 247             | 0.0721  | 0.0739 | 0.0039 | 0.9758 | 0.3301  |
| 16   | rs3764261| 56993324                 | 247             | -0.1114 | 0.0658 | 0.0116 | -1.6940 | 0.0916  |
| 16   | rs12447924| 56994192                | 245             | -0.0866 | 0.0754 | 0.0054 | -1.1490 | 0.2517  |
| 16   | rs4783961| 56994894                 | 247             | -0.0268 | 0.0605 | 0.0008 | -0.4437 | 0.6577  |
| 16   | rs4783962| 56995038                 | 247             | 0.0309  | 0.0788 | 0.0006 | 0.3914 | 0.6959  |
| 16   | rs1800775| 56995236                 | 247             | 0.0567  | 0.0621 | 0.0034 | 0.9127 | 0.3623  |
| 16   | rs1864163| 56997233                 | 242             | -0.0122 | 0.0750 | 0.0001 | -0.1622 | 0.8713  |
| 16   | rs7203984| 56999258                 | 246             | -0.1174 | 0.0590 | 0.0160 | -1.9890 | 0.0478  |
| 16   | rs12597002| 57002404                | 247             | 0.0600  | 0.0692 | 0.0031 | 0.8676 | 0.3864  |
| 16   | rs891142 | 57003977                 | 246             | -0.0751 | 0.1151 | 0.0017 | -0.6527 | 0.5146  |
| 16   | rs12720860| 57004662                | 247             | -0.3030 | 0.2037 | 0.0090 | -1.4880 | 0.1382  |
| 16   | rs7205804| 57004889                 | 241             | -0.0097 | 0.0631 | 0.0001 | -0.1542 | 0.8776  |
| 16   | rs1532624| 57005479                 | 247             | -0.0014 | 0.0633 | 0.0000 | -0.0217 | 0.9827  |
| 16   | rs12708974| 57005550                | 247             | 0.1446  | 0.1023 | 0.0081 | 1.4130  | 0.1588  |
| 16   | rs12720872| 57005882                | 247             | -0.2828 | 0.1833 | 0.0096 | -1.5420 | 0.1243  |
| 16   | rs7499892| 57006590                 | 247             | -0.0666 | 0.0713 | 0.0035 | -0.9338 | 0.3513  |
| 16   | rs9930761| 57007192                 | 247             | 0.2321  | 0.1142 | 0.0166 | 2.0320  | 0.0432  |
| 16   | rs5883  | 57007353                 | 247             | 0.2106  | 0.1218 | 0.0121 | 1.7290  | 0.0850  |
| 16   | rs289714 | 57007451                 | 246             | -0.0752 | 0.0615 | 0.0061 | -1.2220 | 0.2230  |
| 16   | rs12691052| 57007512                | 247             | 0.0782  | 0.1787 | 0.0008 | 0.4374  | 0.6622  |
| 16   | rs289715 | 57008508                 | 247             | -0.0864 | 0.0757 | 0.0053 | -1.1410 | 0.2549  |
| 16   | rs4784744| 57011185                 | 247             | 0.0464  | 0.0627 | 0.0022 | 0.7403  | 0.4598  |
| 16   | rs12720898| 57011243                | 247             | 0.1187  | 0.1088 | 0.0048 | 1.0910  | 0.2764  |
| 16   | rs891144 | 57011936                 | 247             | 0.0548  | 0.1076 | 0.0011 | 0.5094  | 0.6109  |
| 16   | rs12708980| 57012379                | 247             | -0.1229 | 0.0669 | 0.0136 | -1.8370 | 0.0675  |
| 16   | rs7195984| 57015463                 | 247             | -0.0709 | 0.0989 | 0.0021 | -0.7172 | 0.4739  |
| 16   | rs5882  | 57016092                 | 247             | -0.0677 | 0.0586 | 0.0054 | -1.1560 | 0.2487  |
| 16   | rs5742907| 57016150                 | 247             | -0.7002 | 0.6914 | 0.0042 | -1.0130 | 0.3122  |
| 16   | rs12596364| 57016519                | 247             | 0.0216  | 0.1514 | 0.0001 | 0.1425  | 0.8686  |
| 16   | rs289740 | 57016950                 | 244             | -0.0694 | 0.1125 | 0.0016 | -0.6172 | 0.5377  |
| 16   | rs9923854| 57017002                 | 247             | 0.2583  | 0.1044 | 0.0244 | 2.4750  | 0.0140  |
| 16   | rs2228667| 57017279                 | 247             | 0.0936  | 0.6928 | 0.0001 | 0.1350  | 0.8927  |
| 16   | rs2303790| 57017292                 | 245             | -0.2835 | 0.2478 | 0.0054 | -1.1440 | 0.2537  |
| 16   | rs1800777| 57017319                 | 247             | -0.0417 | 0.2336 | 0.0001 | -0.1783 | 0.8586  |
| 16   | rs5887  | 57017552                 | 243             | -0.2041 | 0.2455 | 0.0029 | -0.8316 | 0.4065  |
| 16   | rs1801706| 57017662                 | 247             | 0.2163  | 0.0797 | 0.0292 | 2.7150  | 0.0071  |

DOI:10.1371/journal.pone.0164151.t004
with expression level and patients were having higher frequency of "A". Interestingly, in QTL analysis, we observed that our prediction is true.

It is well known that inhibition of CETP decrease LDL level while increase the HDL, vice-versa might be true. We can predict that high expression of CETP, due to mutant allele "A", is responsible for high LDL level in CAD patients. This might be the reason of plague formation in blood vessels and further causing coronary artery disease.

Further, data obtained for rs1801706 (c.*84G>A) from the 1000 genome project revealed that only 3 populations were having frequency of <0.1; (1) 0.086 in CDX (Chinese Dai), (2) 0.078 in MXL (Mexicans) and (3) 0.029 in PEL (Peruvians) (Table 5). Populations with Indian ancestry (GIH, STU, ITU and BEB) have higher frequency of this allele compared to the controls. This might be due to the admixture of migrant Indians with local populations, who might have higher frequency of rs1801706-A allele (Europeans population in 1000 genome project).
Conclusion

In conclusion, our study revealed rs1801706 (c.*84G>A), a functionally relevant variant in 3’ UTR of CETP, is strongly associated with CAD in South Indian. Interestingly, mutant allele “A” was found to be associated with higher concentration of CETP mRNA. Since, CETP involve in the conversion of HDL to LDL/VLDL, we are tempted to conclude that rs1801706-A increases the risk of CAD by increasing rate of conversion of HDL to LDL/VLDL, through changing the half life of CETP mRNA.

Table 5. Frequency spectrum of allele and genotype of rs1801706/ c.*84G>A in case, control and different world populations.

| Population       | Allele frequency (count) | Genotype frequency (count) |
|------------------|--------------------------|---------------------------|
|                  | Wild type allele "G"     | Mutant allele "A"         | GG            | AG            | AA            |
| Case             | 0.777 (502)              | 0.2229 (144)              | 0.6037 (195)  | 0.3467 (112)  | 0.0495 (16)   |
| Control          | 0.87 (522)               | 0.13 (78)                 | 0.7666 (230)  | 0.2066 (62)   | 0.0266 (8)    |
| All population   | 0.845 (4230)             | 0.155 (778)               | 0.713 (1785)  | 0.264 (660)   | 0.024 (59)    |
| South-Asians     | 0.786 (769)              | 0.214 (209)               | 0.607 (297)   | 0.358 (175)   | 0.035 (17)    |
| BEB (Bengali)    | 0.791 (136)              | 0.209 (36)                | 0.605 (52)    | 0.372 (32)    | 0.023 (2)     |
| GIH (Gujaratian) | 0.801 (165)              | 0.199 (41)                | 0.612 (63)    | 0.379 (39)    | 0.010 (1)     |
| ITU (Telugu)     | 0.775 (158)              | 0.225 (46)                | 0.588 (60)    | 0.373 (38)    | 0.039 (4)     |
| PJL (Punjabi)    | 0.771 (148)              | 0.229 (44)                | 0.583 (56)    | 0.375 (36)    | 0.042 (4)     |
| STU (SriLankan)  | 0.794 (162)              | 0.206 (42)                | 0.647 (66)    | 0.294 (30)    | 0.059 (6)     |
| East-Asians      | 0.895 (902)              | 0.105 (106)               | 0.810 (408)   | 0.171 (86)    | 0.020 (10)    |
| CDX (Chinese Dai)| 0.914 (170)              | 0.086 (16)                | 0.828 (77)    | 0.172 (16)    | 0.0 (0)       |
| CHB (Han Chinese)| 0.883 (182)              | 0.117 (24)                | 0.796 (82)    | 0.175 (18)    | 0.029 (3)     |
| CHS (Southern Han Chinese) | 0.900 (189) | 0.100 (21) | 0.829 (87) | 0.143 (15) | 0.029 (3) |
| JPT (Japanese)  | 0.880 (183)              | 0.120 (25)                | 0.788 (82)    | 0.183 (19)    | 0.029 (3)     |
| KHV (Kinh)       | 0.899 (178)              | 0.101 (20)                | 0.808 (80)    | 0.182 (18)    | 0.010 (1)     |
| African          | 0.846 (1119)             | 0.154 (203)               | 0.717 (474)   | 0.259 (171)   | 0.024 (16)    |
| ACB (Caribbeans) | 0.854 (164)              | 0.146 (28)                | 0.719 (69)    | 0.271 (26)    | 0.010 (1)     |
| ASW (Afro-Americans) | 0.885 (108) | 0.115 (14) | 0.770 (47) | 0.230 (14) | 0.0 (0) |
| ESN (Esan)       | 0.843 (167)              | 0.157 (31)                | 0.707 (70)    | 0.273 (27)    | 0.020 (2)     |
| LWK (Luhya)      | 0.808 (160)              | 0.192 (38)                | 0.657 (65)    | 0.303 (30)    | 0.040 (4)     |
| MAG (Mandinka)   | 0.858 (194)              | 0.142 (32)                | 0.761 (86)    | 0.195 (22)    | 0.044 (5)     |
| MSL (Mende)      | 0.865 (147)              | 0.135 (23)                | 0.741 (63)    | 0.247 (21)    | 0.012 (1)     |
| YRI (Yoruba)     | 0.829 (179)              | 0.171 (37)                | 0.685 (74)    | 0.287 (31)    | 0.028 (3)     |
| American         | 0.905 (628)              | 0.095 (66)                | 0.821 (285)   | 0.167 (58)    | 0.012 (4)     |
| CLM (Colombians) | 0.878 (165)              | 0.122 (23)                | 0.787 (74)    | 0.181 (17)    | 0.032 (3)     |
| MXL (Mexicans)   | 0.922 (118)              | 0.078 (10)                | 0.844 (54)    | 0.156 (10)    | 0.0 (0)       |
| PEL (Peruvians)  | 0.971 (165)              | 0.029 (5)                 | 0.941 (80)    | 0.059 (5)     | 0.0 (0)       |
| PUR (Puerto Ricans) | 0.865 (180) | 0.135 (28) | 0.740 (77) | 0.250 (26) | 0.010 (1) |
| Europeans        | 0.807 (812)              | 0.193 (194)               | 0.638 (321)   | 0.338 (170)   | 0.024 (12)    |
| CEU (Utah residents) | 0.778 (154) | 0.222 (44) | 0.586 (58) | 0.384 (38) | 0.030 (3) |
| FIN (Finnish)    | 0.813 (161)              | 0.187 (37)                | 0.657 (65)    | 0.313 (31)    | 0.030 (3)     |
| GBR (British)    | 0.797 (145)              | 0.203 (37)                | 0.626 (57)    | 0.341 (31)    | 0.033 (3)     |
| IBS (Iberian)    | 0.822 (176)              | 0.178 (38)                | 0.654 (70)    | 0.336 (36)    | 0.009 (1)     |
| TSI (Toscani)    | 0.822 (176)              | 0.178 (38)                | 0.664 (71)    | 0.318 (34)    | 0.019 (2)     |

doi:10.1371/journal.pone.0164151.t005
Supporting Information

S1 Table. Details of normalized expression value of CETP mRNA with genotype of rs1801706/c. **84G>A** in same samples of HapMap populations. (DOCX)

Acknowledgments

We thank all the patients and the physicians, who helped us with this study. KT supported by Network project grant (CardioMed-BSC0122), Council of Scientific and Industrial Research, Government of India, New Delhi, India.

Author Contributions

Conceptualization: KT MG.
Data curation: KT.
Formal analysis: MG SKK.
Funding acquisition: KT.
Investigation: KT.
Methodology: MG SN SKK.
Project administration: KT.
Resources: UKH VKK LL KK.
Software: SN.
Supervision: KT.
Validation: SN.
Visualization: SN.
Writing – original draft: MG SKK SN.
Writing – review & editing: KT.

References

1. Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, et al. (2009) Heart disease and stroke statistics—2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 119: 480–486. doi: 10.1161/CIRCULATIONAHA.108.191259 PMID: 19171871
2. Wang Q (2005) Molecular genetics of coronary artery disease. Curr Opin Cardiol 20: 182–188. PMID: 15861005
3. Zdravkovic S, Wienke A, Pedersen NL, Marenberg ME, Yashin AI, et al. (2002) Heritability of death from coronary heart disease: a 36-year follow-up of 20 966 Swedish twins. J Intern Med 252: 247–254. PMID: 12270005
4. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA (2002) Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 70: 425–434. doi: 10.1086/338688 PMID: 11791212
5. Hopkins PN, Heiss G, Ellison RC, Province MA, Pankow JS, et al. (2003) Coronary artery disease risk in familial combined hyperlipidemia and familial hypertriglyceridemia: a case-control comparison from the National Heart, Lung, and Blood Institute Family Heart Study. Circulation 108: 519–523. doi: 10.1161/01.CIR.0000081777.17879.85 PMID: 12847072
6. Kessler T, Erdmann J, Schunkert H (2013) Genetics of coronary artery disease and myocardial infarction—2013. Curr Cardiol Rep 15: 368. doi: 10.1007/s11886-013-0368-0 PMID: 23616109

7. Consortium IKC (2011) Large-scale gene-centric analysis identifies novel variants for coronary artery disease. PLoS Genet 7: e1002260. doi: 10.1371/journal.pgen.1002260 PMID: 21966275

8. Bruce C, Tall AR (1995) Cholesterol ester transfer proteins, reverse cholesterol transport, and atherosclerosis. Curr Opin Lipidol 6: 306–311. PMID: 8520853

9. Gordon DJ, Rifkind BM (1989) High-density lipoprotein—the clinical implications of recent studies. N Engl J Med 321: 1311–1316. doi: 10.1056/NEJM198911093211907 PMID: 2677733

10. Reich D, Thangaraj K, Patterson N, Price AL, Singh L (2009) Reconstructing Indian population history. Nature 461: 489–494. doi: 10.1038/nature08365 PMID: 19779445

11. Dhandapani PS, Sadayappan S, Xue Y, Powell GT, Rani DS, et al. (2009) A common MYBPC3 (cardiac myosin binding protein C) variant associated with cardiomyopathies in South Asia. Nat Genet 41: 187–191. doi: 10.1038/ng.309 PMID: 19151713

12. Selvi Rani D, Nallari P, Dhandapany PS, Rani J, Meraj K, et al. (2015) Coexistence of Digenic Mutations in Both Thin (TPM1) and Thick (MYH7) Filaments of Sarcomeric Genes Leads to Severe Hypertrophic Cardio-myopathy in a South Indian FHCM. DNA Cell Biol 34: 350–359. doi: 10.1089/dna.2014.2650 PMID: 25607779

13. Shaddhda Chauhan DBTA (2013) Prevalence of cardiovascular disease in India and its economic impact-A review. International Journal of Scientific and Research Publications 3: 5.

14. Hoogeveen RC, Gambhir JK, Gambhir DS, Kimball KT, Ghazzaly K, et al. (2001) Evaluation of Lptabla and other independent risk factors for CHD in Asian Indians and their USA counterparts. J Lipid Res 42: 631–638. PMID: 11290835

15. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215. PMID: 3344216

16. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–575. doi: 10.1086/519795 PMID: 17701901

17. Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, et al. (2007) Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science 315: 848–853. doi: 10.1126/science.1136678 PMID: 17289997

18. Barkowski RS, Frishman WH (2008) HDL metabolism and CETP inhibition. Cardiol Rev 16: 154–162. doi: 10.1097/CRD.0b013e31816a3b60 PMID: 18414186

19. Tan MH (1980) HDL-cholesterol: the negative risk factor for coronary heart disease. Ann Acad Med Singapore 9: 491–495. PMID: 7018364

20. Stranger BE, Nica AC, Forrest MS, Dimas A, Bird CP, et al. (2007) Population genomics of human gene expression. Nat Genet 39: 1217–1224. doi: 10.1038/ng2142 PMID: 17873874

21. Hirano K, Yamashita S, Matsuzawa Y (2000) Pros and cons of inhibiting cholesteryl ester transfer protein. Curr Opin Lipidol 11: 589–596. PMID: 11086331

22. Ganesan M, Bhaskar S, Mani R, Idris MM, Khaja N, et al. (2011) The relationship of ACE and CETP gene polymorphisms with cardiovascular disease in a cohort of Asian Indian patients with and those without type 2 diabetes. J Diabetes Complications 25: 303–308. doi: 10.1016/j.jdiacomp.2010.10.001 PMID: 21185205

23. Ridker PM, Pare G, Parker AN, Zee RY, Miletich JP, et al. (2009) Polymorphism in the CETP gene region, HDL cholesterol, and risk of future myocardial infarction: Genomewide analysis among 18 245 initially healthy women from the Women's Genome Health Study. Circ Cardiovasc Genet 2: 26–33. doi: 10.1161/CIRCGENETICS.108.1817304 PMID: 20031564

24. Papp AC, Pinsonneault JK, Wang D, Newman LC, Gong Y, et al. (2012) Cholesteryl Ester Transfer Protein (CETP) polymorphisms affect mRNA splicing, HDL levels, and sex-dependent cardiovascular risk. PLoS One 7: e31930. doi: 10.1371/journal.pone.0031930 PMID: 22403620

25. Terran-Garcia M, Despres JP, Tremblay A, Bouchard C (2008) Effects of cholesterol ester transfer protein (CETP) gene on adiposity in response to long-term overfeeding. Atherosclerosis 196: 455–460. doi: 10.1016/j.atherosclerosis.2006.12.005 PMID: 17196207

26. Tang ZH, Fang Z, Zhou L (2013) Human genetics of diabetic vascular complications. J Genet 92: 677–694. PMID: 24371189

27. Lu Y, Dolle ME, Imholz S, van ’t Slot R, Verschuren WM, et al. (2008) Multiple genetic variants along candidate pathways influence plasma high-density lipoprotein cholesterol concentrations. J Lipid Res 49: 2582–2589. doi: 10.1194/jlr.M800232-JLR200 PMID: 18660489
28. Moleres A, Milagro FI, Marcos A, Gonzalez Zorzano E, Campoy C, et al. (2014) Common variants in genes related to lipid and energy metabolism are associated with weight loss after an intervention in overweight/obese adolescents. Nutr Hosp 30: 75–83. doi: 10.3305/nh.2014.30.1.7542 PMID: 25137265

29. Talmud PJ, Drenos F, Shah S, Shah T, Palmen J, et al. (2009) Gene-centric association signals for lipids and apolipoproteins identified via the HumanCVD BeadChip. Am J Hum Genet 85: 628–642. doi: 10.1016/j.ajhg.2009.10.014 PMID: 19913121