Effect of genetic variant (rs11887534) in \(\text{ABCG8}\) gene in coronary artery disease and response to atorvastatin therapy

Anshika Srivastava\(^a\), Naveen Garg\(^b\), Anvesha Srivastava\(^a\), Kshitij Srivastava\(^a\) and Balraj Mittal\(^a\),\(^*\)

\(^a\)Department of Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, India
\(^b\)Department of Cardiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, India

Abstract. Background: ATP-binding cassette transporter ABCG8 plays an important role in excretion of cholesterol from liver. Common genetic polymorphisms in \(\text{ABCG8}\) gene may genetically predispose an individual to coronary artery disease (CAD) along with response to atorvastatin therapy. Thus, we aimed to examine the role of \(\text{ABCG8}\) D19H polymorphism (rs11887534) in susceptibility to CAD and its influence on atorvastatin response.

Methodology: The study included 213 CAD patients and 220 controls. Genotyping of \(\text{ABCG8}\) D19H polymorphism was done by PCR-RFLP.

Results: Our results showed that \(\text{ABCG8}\) ‘\(H\)’ allele was conferring significant risk for CAD in a dominant model (OR = 2.54; \(p = 0.014\)). This increased risk for CAD was more pronounced in males (OR = 2.69; \(p = 0.030\)). No correlation of \(\text{ABCG8}\) genotypes with the risk factors (diabetes, hypertension and smoking) of CAD was observed. On atorvastatin treatment there was a significant decrease in the LDL-C levels (\(p = 0.021\)). However, stepwise multiple regression analysis showed that this decrease was not associated with \(\text{ABCG8}\) genetic variant (\(p = 0.845\)). Observed determinants of variation in interindividual response to atorvastatin therapy were pre-treatment LDL-C (\(p = 0.024\)) and TC (\(p = 0.017\)).

Conclusion: Although the genetic variant 19H of \(\text{ABCG8}\) confers risk for CAD in North Indian population, it is not associated with interindividual response to atorvastatin therapy.

Keywords: Coronary artery disease, polymorphism, \(\text{ABCG8}\), Atorvastatin therapy, PCR-RFLP

1. Introduction

Coronary artery disease (CAD) is the most common cause of fatality, disability and economic loss, particularly in industrialized nations. The major cause of CAD is underlying atherosclerosis (arteriosclerosis) which leads to the formation of atherosclerotic plaques within the arteries [6,13]. The plaques are characterized by accumulation of fibrous tissue, cell components (mostly macrophages, smooth muscle cells, and lymphocytes) and plasma lipids [17,18,21]. CAD is believed to be caused by the interactions between various genetic and environmental factors.

Increased plasma cholesterol level is an established risk factor for coronary artery disease. Role of various genes related to cholesterol homeostasis have been previously studied and some of them have been shown to be associated with risk of CAD [1,14,15,22]. In recent years, genes such as \(\text{ABCG5/8}\) have attained great importance due to their role in cholesterol synthesis and transport [5,11].

ATP binding cassette transporters G5 and G8 (\(\text{ABCG5/8}\)) are unique proteins present in the plasma membrane, as well as intracellular membranes of en-
terocytes and hepatocytes, where they limit the intestinal absorption of dietary sterols and biliary excretion of sterols. Mutations in either of the two genes that encode these transporters have been reported to cause sitosterolemia (rare autosomal recessive disorder) [4, 23], characterized by elevated plasma levels of plant sterols. Till date, several mutations and polymorphisms have been identified in ABCG5 and ABCG8 genes [2, 12], but of all, D19H polymorphism (rs11887534) in ABCG8 has been significantly associated with several cholesterol related diseases [5,19]. Recently, various studies have reported that ABCG8 D19H polymorphism is significantly and independently associated with a greater LDL cholesterol reduction after atorvastatin treatment, which further supports the possibility that this variant to be associated with gain of function of ABCG8 protein [9].

Looking into the importance of ABCG8 transporter, we hypothesized that presence of common genetic variants in ABCG8 gene may play a role in the susceptibility to coronary artery disease. We therefore explored the relationship between D19H polymorphism in ABCG8 gene and its association with CAD. Furthermore, we also looked for association between ABCG8 D19H polymorphism and response to atorvastatin therapy.

2. Method

2.1. Study design and study population

The present case-control study comprised of 213 patients with significant coronary artery disease, (diagnosis, confirmed by coronary angiography and further these subjects underwent post angioplasty) recruited from the Department of Cardiology of Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, Uttar Pradesh, India. The detailed clinical history of CAD patients was based on hospital investigations including angiography. All the CAD patients were started with 10 mg of atorvastatin/day, with a target of LDL cholesterol 70 mg/dl. None of the patients were familial hypercholesterolemic cases. A total of 220 healthy controls were recruited from unrelated individuals of North India. The inclusion criteria for controls were absence of prior history of high systolic blood pressure, family history of CAD, diabetes mellitus, and obesity. After obtaining informed consent, all the individuals were personally interviewed for information on ethnicity, food habits, occupation and tobacco usage. Both patients and controls had similar ethnicity. The study was approved by local ethics committee of the institute. Blood samples from all the subjects were collected in EDTA and stored at −70°C

2.2. Data collection

Data was obtained by reviewing the patient’s medical records. Hypertension was defined as systolic blood pressure > 140 mmHg or a diastolic blood pressure > 90 mmHg or patients using antihypertensive drugs. Smoking was classified as smokers (ex-smoker and current smokers) and non smokers. Similarly, diabetes mellitus was defined as patients with fasting plasma glucose > 6.9 mmol/L or patients using anti-diabetic medication. All laboratory parameters, as stated in the medical record, were determined in fasting patients. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride levels were measured by standard enzymatic methods. LDL cholesterol concentrations were calculated using the Friedewald formula [8].

2.3. Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes according to a standard salting out method [16]. Laboratory personnel were blinded to the case – control status of the subjects. D19H polymorphism in ABCG8 gene loci was determined using standard polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The ABCG8 D19H polymorphic site containing fragment was amplified by PCR as described by Stefkova et al. [20]. The G>C transversion at the polymorphic site of ABCG8 gene creates a BamHI restriction site. The PCR product was digested with respective restriction enzyme (New England Biolabs Inc., Beverly, MA, USA) and the digestion product was analyzed on 15% polyacrylamide gel. The D allele (homozygous wild) was characterized by the presence of two digested fragments of 56 and 27 bp whereas the H (homozygous variant) allele remained undigested. Genotypes of 50% samples were confirmed by Taqman assay. Taqman SNP Genotyping Assay ID for the D19H polymorphism was C26135643 10 (Applied Biosystems). Reaction components and amplification parameters were based on the manufacturer’s instructions.

2.4. Statistical analysis

Descriptive statistics of patients and controls were presented as mean and standard deviation (SD) for continuous measures while frequencies and percentages were used for categorical measures. The chi-square goodness of fit test was used for any deviation
from Hardy Weinberg Equilibrium in controls. Differences in genotype and allele frequencies between study groups were estimated by chi-square test. CAD risk in relation to ABCG8 D19H genotypes was estimated by using unconditional logistic regression to calculate odds ratios (OR) and 95% confidence intervals. The ORs were adjusted for confounding factors such as age and gender. Since no individual was found to be homozygous for the ABCG8 polymorphism, we combined heterozygous and homozygous carriers of the minor allele (dominant model). Differences in plasma cholesterol concentrations and patient characteristics among ABCG8 genotypes were analyzed with chi-square statistics. Multiple linear regression analysis was used to adjust statistical tests for the effects of age and gender. Moreover for confirmation, whether variant (D19H) genotype contributes to significant lowering of LDL-C levels we performed independent t-test. A two-tailed \( p \)-value of less than 0.05 was considered a statistical significant result. All statistical analyses were performed using SPSS software version 15.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Patient characteristics and genotype frequencies

Characteristics of CAD patients are shown in Table 1. The mean age of CAD patients and healthy controls was 56.73 ± 9.9 and 54.95 ± 8.1. In patients 81.3% subjects were males while 75% of the controls were males. In CAD patients 44.6 were hypertensive and 29.6% were diabetic while 46.0% were smokers. Patients with stable angina and unstable angina were 30.5%, 13.6% respectively. Patients with Non ST Segment Elevation Myocardial Infarction (NSTEMI) were 9.4%. Under ST Segment Elevation Myocardial Infarction (STEMI) patients with anterior wall myocardial infarction (AWMI) were 23.9% and 18.3% respectively, while no patient was affected with lateral wall myocardial infarction. Atypical chest pain and coronary artery bypass surgery (CABG) formed the 4.3% of the total patients which were combined together as others.

The angiographic profile categorized patients with single vessel disease (SVD), double vessel disease (DVD), and triple vessel disease (TVD) as 58.2%, 33.8% and 8% respectively.

| Characteristics | N = 213 |
|-----------------|--------|
| Mean age ± SD   | 56.73 ± 9.9 |
| Male sex – No%  | 173 (81.3) |
| Hypertension – No% | 95 (44.6) |
| Diabetic – No%  | 63 (29.6) |
| Smokers – No%   | 98 (46.0) |

**Clinical syndrome**

- a) Stable angina – No% 65 (30.5)
- b) Unstable angina – No% 29 (13.6)
- c) Non ST segment elevation myocardial infarction (NSTEMI) – No% 20 (9.4)
- d) ST segment elevation myocardial infarction STEMI
  - i. Anterior wall myocardial infarction (AWMI) – No% 51 (23.9)
  - ii. Inferior wall myocardial infarction (IWMI) – No% 39 (18.3)
- e) Others – No% 9 (4.3)

**Angiographic profile**

- Single vessel disease (SVD) – No% 124 (58.2)
- Double vessel disease (DVD) – No% 72 (33.8)
- Triple vessel disease (TVD) – No% 17 (8)

3.2. Frequency distribution of genotypes and alleles in CAD patients and controls

The observed genotype distribution of ABCG8 D19H polymorphism in healthy controls was consistent with Hardy–Weinberg equilibrium. Among healthy controls, the frequencies of the wildtype (D) and variant (H) alleles were 0.95 and 0.5 respectively. On comparing the genotype frequency distribution in CAD patients with that of healthy controls, the frequency of the heterozygous DH genotype was considerably higher (11.3%) in patients than that in controls (5.0%). This difference in frequency was statistically significant (\( p = 0.014 \)) and was conferring high risk for CAD (OR = 2.54; 95% CI = 1.2–5.3) (Table 2).

At allele level also there was an increased distribution of H allele in CAD patients in comparison to healthy controls (5.5% vs 2.5%) and this difference was also statistically significant (\( p=0.017 \)) and conferring high risk for the disease (OR = 2.44; 95% CI = 1.1–5.0) (Table 2).

After stratification of subjects on the basis of gender, there was a significant increased risk for CAD in males in presence of DD/DH genotype (\( p = 0.027 \); OR = 2.79; 95% CI = 1.1–6.9) and H allele (\( p = 0.030 \); OR = 2.69; 95% CI = 1.1–6.5). Although there was also an increased risk for female CAD patients (OR = 2.24 and 2.15 respectively for DH/HH genotype and H allele), but it was not statistically significant (Table 2).
3.3. Influence of ABCG D19H polymorphism on CAD risk by established risk factors

A case only study was performed to estimate the association between the genotypes of ABCG8 polymorphism and the established risk factors for coronary artery disease. All the CAD patients were divided on the basis of associated disease such as diabetes mellitus, hypertension and smoking status of the subjects and were compared for the distribution of ABCG8 genotype in these groups. However, ABCG8 D19H polymorphism was not found to modulate the CAD risk by diabetes, hypertension or smoking status of the patients (Table 3).

3.4. Influence of ABCG8 polymorphism on lipid profile

No significant difference in baseline plasma lipid concentrations among individuals carrying D19H genotypes was observed. However, post-treatment TC values of subjects having at least one variant allele were significantly lower ($p = 0.041$) than those of wild type allele homozygotes. On calculating the percentage reduction of LDL-C and TC, percent reduction of LDL-C and TC levels after treatment with statin (Table 4).

3.5. Identification of independent risk factors associated with CAD risk

To identify independent variables associated with atorvastatin effects, stepwise multiple regression analysis was performed including age, gender, pretreatment lipid levels (LDL-C, and HDL Cholesterol), and each genotype as independent variables. This analysis showed that pretreatment LDL-C and TC levels ($p = 0.024$ and $0.017$ respectively) were significantly and independently associated with post-treatment LDL-C level. However, in our population D19H variant genotype was not associated independently with the absolute and percent reduction of LDL-C ($p = 0.845$) (Table 5).

4. Discussion

In the present study, we examined the possible association between common polymorphism D19H in ABCG8 with CAD, which has been suggested to play major role in cholesterol excretion from the liver into bile. Also we elucidated the role of this genetic variation in conferring interindividual variation in response to statin therapy. Main outcomes of this study are: (1) D19H genetic variation in ABCG8 polymorphism has the potential to influence the risk of coronary heart disease, (2) D19H polymorphism does not influence significantly the decrease in total LDL cholesterol from statin therapy and (3) instead, pre-treatment LDL-C and TC levels are the major determinant of the interindividual variation in response to statin therapy.

It has been suggested that common DNA sequence variations in the ABCG8 contributed to the variation in plasma concentrations of sterols [3,5]. In addition, ABCG8 variants were also associated with plasma total cholesterol and low-density lipoprotein (LDL) cholesterol levels in some studies [3,7,9]. In our case-control study, we also observed a significant risk for CAD in the subjects carrying the variant 19H allele of ABCG8. A recent study by Koeijvoets et al. [10] also showed that individuals carrying the risk genotype for ABCG8 variants had an increased risk of CVD and CHD.

Although, it was speculated that substitution of histidine for aspartic acid at 19th amino acid increases the transporter function of ABCG8. Resultant enhanced efflux of sterols from enterocytes to small intestinal lumen and augmented excretion of sterols from the liver into the bile would lead to lower plasma sterol concentrations which should be rather protective for CAD risk. On the other hand, our results are contrary to the estab-
Table 3
Correlation of ABCG8 genotypes with the risk factors of coronary artery disease

| Risk factors       | Genotypes | Frequency (%) | OR (95% CI) | p-value |
|--------------------|-----------|---------------|-------------|---------|
| Diabetes mellitus  | DD        | 134 (89.3)    | 0.757 (0.3–1.8) | 0.553   |
|                    | DH/HH     | 16 (10.7)     |             |         |
| Diabetic (63)      | DD        | 55 (87.3)     |             |         |
|                    | DH/HH     | 8 (12.7)      |             |         |
| Hypertension       | DD        | 134 (91.2)    |             |         |
|                    | DH/HH     | 13 (8.8)      |             |         |
| Smoking            | DD        | 105 (91.3)    | 1.83 (0.7–4.3) | 0.172   |
|                    | DH/HH     | 10 (8.7)      |             |         |

**Note:** 
- **OR**: Odds ratio, 
- **CI**: Confidence interval.

Table 4
Lipid and lipoprotein concentration before and after atorvastatin treatment according to D19H polymorphism (rs11887534) of ABCG8

| All subjects | DD | DH/HH | P-value |
|--------------|----|-------|---------|
| No of subjects | 151 | 134 | 17 |
| Age (years)  | 56.81 ± 10.00 | 57.20 ± 10.11 | 53.71 ± 8.76 | 0.176 |
| Baseline (mg/dl) | 148.91 ± 33.11 | 147.13 ± 34.21 | 162.88 ± 17.73 | 0.065 |
| TC | 140.65 ± 30.88 | 89.57 ± 30.54 | 99.12 ± 33.19 | 0.231 |
| LDL-C | 32.16 ± 7.64 | 32.07 ± 7.96 | 32.82 ± 4.61 | 0.705 |
| HDL-C | 34.04 ± 7.71 | 33.99 ± 8.01 | 34.41 ± 4.43 | 0.834 |
| Treatment (mg/dl) | 141.89 ± 39.56 | 139.55 ± 39.87 | 160.35 ± 32.41 | 0.041 |
| TC | 76.71 ± 26.83 | 75.25 ± 26.76 | 88.18 ± 25.33 | 0.061 |
| LDL-C | 34.04 ± 7.71 | 33.99 ± 8.01 | 34.41 ± 4.43 | 0.834 |
| HDL-C | 13.64 ± 19.27 | 14.92 ± 15.42 | 3.51 ± 37.15 | 0.021 |
| %Change (adjusted) | -7.13 ± 16.64 | -7.38 ± 17.50 | -5.15 ± 6.79 | 0.605 |

Significant values are represented in bold.

Table 5
Stepwise multiple regression coefficients for the post treatment LDL-C level by atorvastatin

| Variables      | Coefficient | SE * | p-value |
|----------------|-------------|------|---------|
| Pretreatment LDL-C | 0.146        | 0.065 | 0.024   |
| Age            | 0.033       | 0.051 | 0.522   |
| D19H variant   | 0.269       | 1.375 | 0.845   |
| Pretreatment TC | 0.106       | 0.045 | 0.017   |

*SE*: Standard error.
Significant values are represented in bold.

While this notion is published but consistent with reports by Koeijvoets et al. [10] and support the risk for CAD in the presence of ABCG8 variant. The physiological effect behind the increased risk of CAD in the presence of genetic variant of ABCG8 is not very clear. The effect of D19H as a gain of function has been derived from studies in vitro, however, there are no reports determining the effect of this genetic variation on ABCG8 function in an in vitro assay or in knock-in mouse models. Therefore, there is a need to re-evaluate the function of D19H variation in vivo. In addition, possibility of other, yet unknown, factors responsible for the observed results cannot be ruled out. We observed a significant risk for CAD in male patients which may be the reflection of larger sample size in this subgroup. Results showed that the risk for CAD in the presence of 19H variant was not correlated with other risk factors of CAD such as diabetes, hypertension and smoking status of the subjects.

To test if the genetic variation in ABCG8 might influence the plasma lipid response to statin therapy, we examined the role of ABCG8 D19H polymorphism in 213 CAD patients treated with 10 mg atorvastatin. Our results suggested that there is a significant reduction in LDL-C level in the carriers of ABCG8 19H allele. However, stepwise multiple logistic regression analysis revealed that this decrease in LDL-C on atorvastatin treatment is not independently associated with the ABCG8 genotype which was further confirmed by independent sample t test. However, Kajinami et al. [16] reported that ABCG8 D19H polymorphism was significa-
cantly and independently associated with a greater LDL cholesterol reduction after atorvastatin treatment. Major determinants of the atorvastatin therapy response observed in our study were pre-treatment LDL-C and TC. This observation is consistent with the various reports which also suggest that pre-treatment LDL-C level is the main determinant of the response to the statin therapy with little benefit occurring in patients with average baseline levels [22,23]. Kajinami et al. [9] also suggests that determinants of steady-state LDL cholesterol levels may differ from those of responses to statin treatment. However, there are contradictory reports which associated the ABCG8 genetic variant with greater LDL-C lowering in response to atorvastatin therapy [3,9]. This difference in the observations may be due to the population difference. The frequency of D19H polymorphism is lower in Asians than Caucasians. It is also worth mentioning that in most of the studies patients recruited were hypercholesterolemic, while the patients included in the present study were non-hypercholesterolemic.

In conclusion, our results mirrored that carrier of ABCG8 19H allele are associated with increased risk of CAD but not with plasma cholesterol lowering in response to atorvastatin therapy. Furthermore, pre-treatment LDL-C and TC are the major factors determining the response to atorvastatin therapy.

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