

## Genetic predisposition of E-selectin gene (S128R) polymorphism in patients with coronary artery disease (CAD)

Rajneesh Tripathi, Prabhat Kumar Singh\*, Satyendra Tewari\*\*, Parag M. Tamhankar\*, Venkataraman Ramesh<sup>+</sup> & Sarita Agarwal

*Departments of Medical Genetics, \*Anaesthesiology, \*\*Cardiology & +Clinical Chemistry (Pathology) Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India*

Received May 1, 2008

**Background & objectives:** A surface glycoprotein molecule, E-selectin is involved in adhesion of circulating leukocyte to the activated endothelium and plays a fundamental role in pathogenesis of atherosclerosis. The present study was undertaken to document the status of S128R polymorphism of E-selectin gene in angiographically proven coronary artery disease (CAD) patients from Uttar Pradesh.

**Methods:** Genotype of the S128R polymorphism was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 329 angiographically proven CAD patients [n=83 acute myocardial infarction (AMI) and n= 246 AMI-free] and 331 age and sex matched control individuals (angiographically proven not to have CAD).

**Results:** This pilot study revealed a significant association of R allele in coronary artery disease patients in univariate analysis [allele frequency 9.6% in patients vs. 5.6% in control ( $P = 0.031$ , OR = 1.57, 95% CI = 1.05 – 2.47)]. However, after binomial logistic regression the significant determinants of CAD were: presence of diabetes (OR: 2.26,  $P=0.001$ ) hypertension (OR = 2.61,  $P=0.001$ ), smoking habit (OR=2.038,  $P=0.001$ ), elevated serum triglycerides (OR=1.967,  $P=0.001$ ) and low HDL-C (high density lipoprotein cholesterol) (OR=1.107,  $P=0.001$ ).

**Interpretation & conclusions:** The interaction of classical risk factors for CAD with S128R polymorphism in our study population showed that the significant determinants of coronary artery disease were presence of diabetes, hypertension, smoking habit, elevated serum triglycerides and low HDL. S128R polymorphism in E-selectin gene was not an independent predictor of CAD in our population.

**Key words** Coronary artery disease - E-selectin - genetic predisposition - S128R polymorphism

E-selectin (CD62E) is surface glycoprotein molecule involved in adhesion of circulating leukocyte in activated endothelium and plays an important role in inflammation process. Inflammation is one of the earliest events in pathogenesis of atherosclerosis. E-selectin belongs to a family of structurally related selectin molecule; selectins E, P and L participate in

endothelial leukocyte adhesion<sup>1</sup>. Experiments using E and P-selectin double knockout mice suggest that E-selectin and P-selectin together play an important role in both early and advanced stages of atherosclerotic lesion development<sup>2</sup>. The implication of E-selectin gene in the pathogenesis of coronary artery disease (CAD) has been studied extensively in several ethnic groups

across the globe<sup>3-7</sup>. However, no data are available on association of this polymorphism with CAD in the Indian population where it has a high prevalence<sup>8</sup>. Hence we took up a pilot study to evaluate the status of E-selectin S128R polymorphism in angiographically proven CAD patients in a tertiary care centre in north India and compared the allele frequencies between controls and CAD patients.

### Material & Methods

*Study subjects:* The patients having the evidence of more than 50 per cent stenosis in coronary arteries or history of prior angioplasty or by pass grafting of coronary artery disease (CAD) were included in the present study. The patients were randomly recruited from the outpatient and inpatient services of the Department of Cardiology, Sanjay Gandhi Post-graduate Institute of Medical Sciences (SGPGIMS), Lucknow, from February 2006 to November 2008. The study group included angiographically proven patients as having severe CAD (stenosis > 50%). The controls were individuals who underwent angiography for their cardiac valvular heart diseases and were proven not to have CAD. The two groups were age and sex matched. The classical risk factors such as presence of diabetes, hypertension, smoking habit, family history of CAD, body mass index and complete lipid profile (serum triglycerides, total cholesterol, HDL, LDL and VLDL) noted in both patient and control groups. Diabetes and hypertension were diagnosed in patients and controls on the basis of previous medical records or standard clinical examination and tests as per standard definition<sup>9</sup>. Presence of smoking habit was noted and family history of CAD was defined on the basis of presence of CAD in first degree relatives. Body mass index (BMI) was calculated by dividing weight in kilogram by square of height in meters<sup>9</sup>. Patients and controls included in the study were natives of Uttar Pradesh. Patients from other State were excluded from the study. The prior written informed consent was obtained from all the patients and controls. The study protocol was approved by our institutional ethical committee.

*DNA preparation and genotyping:* Genomic DNA was extracted from EDTA peripheral blood leukocytes by standard phenol-chloroform method<sup>3</sup>. The quality of the DNA was checked on 0.8 per cent agarose (SRL Pvt Ltd, India) gel electrophoresis and quantification was done by UV spectrophotometer (Specgene Ltd., UK) before doing genotyping. Genotyping of the

S128R polymorphism of E-selectin gene was done by PCR-restriction fragment length polymorphism (RFLP). Synthetic oligonucleotides (Bangalore Genei Ltd., Bangalore) for polymerase chain reaction of exon 4 of E-selectin gene were designed as per Li *et al*<sup>10</sup>. Amplification was done in automated thermocycler (BioRad PTC100, USA). The amplified PCR products of 186 bp were digested with Pst I (NEB UK) restriction enzyme as per manufacturer's specifications. The digested products were electrophoresed on 3 per cent agarose. The undigested 186 bp band indicated mutation in E-selectin gene. The genotyping results were confirmed by testing in triplicate.

*Biochemical investigation:* Fasting venous blood from all the patients and control subjects were collected in plain vial for the analysis of triglyceride (TG) levels by lipoprotein lipase-peroxidase method<sup>11</sup>, total cholesterol (TC) levels by cholesterol oxidase method<sup>12</sup> and high density lipoprotein (HDL) levels by phosphotungstate magnesium chloride method<sup>13</sup>. Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) levels were calculated indirectly by Friedwald formula<sup>14</sup> as  $VLDL-C = TG/5$  provided  $TG < 400$  mg/dl and  $LDL-C = TC - (HDL-C + VLDL-C)$ .

*Statistical analysis:* Genotype and allele frequencies in CAD and control groups were compared by Chi square test. The relationship between E-selectin genotypes and biochemical findings in the patients groups were evaluated by student t-test. Additionally, multiple logistic regression models involving the interaction of the genotype and different classical CAD risk factors were performed. The patient group was divided into two groups: those with CAD < 45 yr of age were classified as having premature CAD whereas those with CAD at age >45 yr were classified as having later onset of CAD<sup>15</sup>. All analyses were performed using SPSS v.11.5 (SPSS Inc., Chicago, USA) statistical analysis software. A two-tailed  $P < 0.05$  was considered statistically significant.

### Results & Discussion

The study group included 329 patients [83 having myocardial infarction (AMI) and 246 free from MI] and the control group included 331 individuals. The clinical and demographic data of the patients and control subjects are given in Table 1. The classical risk factors *viz.* diabetes, hypertension, smoking habit ( $P < 0.001$ ) and family history ( $P < 0.05$ ) were significantly higher in CAD group than in the control groups. The values of HDL- cholesterol

is significantly lower in coronary artery disease patients than in controls ( $P<0.05$ ) however, serum triglyceride, total cholesterol ( $P<0.001$ ), LDL-cholesterol ( $P<0.05$ ), VLDL-cholesterol ( $P<0.001$ ) were found significantly higher in patients. The allele and genotype frequencies in patient and control groups are described in Table II. The characteristics of CAD patients were analyzed with respect to E-selectin genotype and statistically insignificant differences were found between genotype and CAD risk factors (Table III). Multiple logistic regression analysis was done for the interaction between E-selectin genotypes and other classical risk factors for CAD (Table IV). Patients having premature CAD were 13.1 per cent (43/329). The percentage of premature CAD patients who had R allele were 20.9 per cent (9/43) whereas, 16.43 per cent (47/286) of patients with later onset of CAD had R allele. Premature CAD had no significant association with S128R polymorphism [ $P=0.513$ , OR 1.247 95% CI (0.674-2.408)].

**Table I.** Characteristics of patient and control groups

| Characteristics                   | Control       | CAD patient                 |
|-----------------------------------|---------------|-----------------------------|
| N                                 | 331           | 329                         |
| Age (yr)                          | 53.18±9.18*   | 57.09±9.66*                 |
| Sex (M:F)                         | 245:86        | 260:69                      |
| BMI (kg/m <sup>2</sup> )          | 25.12±3.12*   | 25.54±2.97*                 |
| Diabetic: non diabetic            | 82:249        | 142:187 <sup>++</sup>       |
| Hypertensive: Non hypertensive    | 76:255        | 156:173 <sup>++</sup>       |
| Smoker: Non smoker                | 33:298        | 66:263 <sup>++</sup>        |
| Positive F/H : Negative F/H       | 04:327        | 13:316 <sup>+</sup>         |
| Triglyceride (45-150 mg/dl)       | 95.75±27.95*  | 165.04±81.33 <sup>***</sup> |
| Total cholesterol (125-250 mg/dl) | 154.70±39.73* | 170.29±48.56 <sup>***</sup> |
| HDL-cholesterol (23-60 mg/dl)     | 38.34±7.79*   | 36.51±7.01 <sup>**</sup>    |
| LDL-cholesterol (92-148 mg/dl)    | 97.82±31.72*  | 103.99±43.67 <sup>**</sup>  |
| VLDL-cholesterol (10-30 mg/dl)    | 19.59±6.65*   | 29.80±13.90 <sup>**</sup>   |

\*Mean±SD,  $P^+<0.05$   $^{++}<0.001$  compared with controls; F/H-family history

**Table II.** E-selectin allele and genotype distribution in CAD patient and control subjects

| Genotype/ allele | Control (n=331)<br>No. (%) | Patient (n=329)<br>No. (%) | P value | OR, (95% CI)      |
|------------------|----------------------------|----------------------------|---------|-------------------|
| SS               | 294 (88.8)                 | 273 (82.9)                 |         |                   |
| SR               | 37 (11.2)                  | 56 (17.1)                  | 0.030   | 1.63, (1.02-2.61) |
| S allele         | 625 (94.4)                 | 602 (91.4)                 |         |                   |
| R allele         | 37 (5.6)                   | 56 (9.6)                   | 0.031   | 1.57, (1.05-2.47) |

**Table III.** Clinical and biochemical values in CAD patients with selectin genotypes

| Characteristics                   | Genotype      |               |
|-----------------------------------|---------------|---------------|
|                                   | S/S (n=273)   | S/R (n=56)    |
| Age                               | 57.42 ±9.65*  | 55.48±9.63*   |
| Sex (M:F)                         | 214 : 59      | 46:10         |
| Diabetic : Non diabetic           | 159 :114      | 28:28         |
| Hypertensive : Non hypertensive   | 146 :127      | 27:29         |
| Smoker : Non smoker               | 225:48        | 27:29         |
| Positive FH : Negative FH         | 264 : 9       | 52 : 4        |
| Triglyceride (45-150 mg/dl)       | 163.63±80.06* | 171.91±87.74* |
| Total cholesterol (125-250 mg/dl) | 168.15±47.20* | 180.73±53.99* |
| HDL-cholesterol (23-60 mg/dl)     | 36.47±7.073*  | 36.71±6.76*   |
| LDL-cholesterol (92-148 mg/dl)    | 101.96±42.65* | 113.89±47.71* |
| VLDL-cholesterol (10-30 mg/dl)    | 29.39±13.37*  | 31.80±16.37*  |

\*Mean±SD

**Table IV.** Multivariate binomial logistic regression analyses to determine predictors of CAD

| Variable              | Odds ratio | 95 % CI      | P value |
|-----------------------|------------|--------------|---------|
| Diabetes              | 2.265      | 1.441-3.712  | 0.001   |
| Smoking               | 2.038      | 1.147-3.623  | 0.001   |
| Hypertension          | 2.615      | 1.667-4.102  | 0.001   |
| Family history of CAD | 2.486      | 0.503-12.284 | 0.265   |
| Triglycerides         | 1.967      | 1.954-0.979  | 0.001   |
| Total cholesterol     | 0.998      | 0.980-1.017  | 0.859   |
| HDL-cholesterol       | 1.107      | 1.067-1.148  | 0.001   |
| LDL-cholesterol       | 0.988      | 0.970-1.007  | 0.226   |
| VLDL-cholesterol      | 1.021      | 0.961-1.085  | 0.496   |
| E-selectin genotype   | 1.487      | 0.839-2.637  | 0.174   |

The implication of E-selectin gene in the pathogenesis of coronary artery disease has been studied extensively in several ethnic groups. In our study, The R allele was significantly higher in the patient group (9.6 %) than in control group (5.6%) ( $P = 0.031$ , OR = 1.57, 95% CI 1.05-2.47). Our findings were in concordance with the earlier studies. Ye *et al*<sup>4</sup> reported that R allele (19.5% in CAD patients versus 10.6% in controls) was significantly associated ( $P<0.05$ ) with CAD in white Americans. Yoshida *et al*<sup>5</sup> found significantly higher R allele frequency (12.5%) in patient group than in control group (6.7%) in Japanese population ( $P<0.001$ , OR=2.0, CI=1.04-3.85). Li *et al*<sup>10</sup> found significantly higher frequency of R allele in patients (6.7%) with CAD versus controls (3.1 %) in Central China (OR= 2.21; 95%CI: 1.20-4.07). Frequency of R allele in CAD patients of Saudi Arabia was also higher in patients

(11%) versus controls (6%) (OR=1.76, 95%CI: 1.14-2.42,  $P=0.007$ )<sup>6</sup>. The frequency of R allele was also the second highest in people of South Asian origin (7.9%) versus whites (9.6%) and people of African origin (3.7%) in a multi-ethnic study from UK<sup>7</sup>.

The frequency of SR genotype was significantly higher in CAD patients (17.1 %) versus SS genotype (11.2%) (OR = 1.63, 95% CI 1.02-2.61,  $P = 0.030$ ). This was similar to study in China where frequency of SR genotype was significantly higher in CAD patients (13.3%) versus control (6.2 %) (OR = 2.3, 95%CI: 1.23-4.3,  $P=0.008$ )<sup>10</sup>. The study conducted in Japan showed significantly higher frequency of SR genotype in CAD patients (12.6 %) compared to that of controls (6.7%) (RR= 2, 95%CI: 1.04-3.85,  $P=0.04$ )<sup>5</sup>. The study in Saudi Arabia reported higher frequency of SR genotype in CAD patients (16.6%) than in controls (11.8%) (OR= 1.52, 95%CI: 0.94 – 2.45  $P=0.07$ )<sup>6</sup>, though the percentage frequency of R allele and SR genotype varies in different ethnic groups but show a significantly higher prevalence trend in patients than controls.

We did not find R/R genotype in Indian population which is in concordance with other studies performed in Chinese<sup>10</sup> and Japanese population<sup>5</sup>. However, a study from Saudi Arabia<sup>6</sup> indicated a significantly higher frequency of RR genotype in CAD patients (2.3 %) compared to controls (0.4 %) (OR= 6, 95%CI: 0.8 – 123.6,  $P = 0.05$ ). According to Hardy Weinberg equilibrium the frequency of R/R genotype expected in our population is very low (0.03). Therefore, it may be due to environmental factors which are selective over the R/R genotype.

The frequencies of classical CAD risk factors such as obesity, hypertension, smoking, family history of CAD, lipid levels like total cholesterol, triglycerides, LDL, VLDL were higher in patients than in controls (Table I) but these were not statistically different between SS and SR genotypes (Table II). These results were similar to that observed in Chinese population<sup>10</sup>.

After multivariable logistic regression analysis the final significant variables in the equation to determine presence of CAD were presence of diabetes, smoking habit, hypertension, serum triglyceride levels and HDL (Table IV). However, presence of family history of CAD, serum total cholesterol, LDL, VLDL and SR genotype were insignificant variables for CAD. Thus the S128R allele was significant predictor of CAD in univariate analysis but lost its association in multivariate analysis. This was similar to results found

in Arabs<sup>6</sup> but contrasted study in Chinese<sup>10</sup> population where SR genotype was one of the final significant variables in multivariable logistic regression analysis.

In conclusion, the interaction of classical risk factors for CAD with S128R polymorphism in our study population showed that the presence of diabetes, hypertension, smoking habit, elevated serum triglycerides and low HDL were significant determinants of coronary artery disease. The other classical risk factors such as presence of family history of CAD, serum total cholesterol, LDL, VLDL and S128R polymorphism in E-selectin gene were not found to be significant determinants of coronary artery disease in our study population. Further studies are required to confirm these findings.

### Acknowledgment

The authors acknowledge the Indian Council of Medical Research, New Delhi, India for providing financial support and Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS) for providing the infrastructure. Authors thank Dr Uttam Singh, Department of Biostatistics, SGPGIMS, for provide statistical support. The first author (RT) was a fellow of Department of Biotechnology (DBT), New Delhi, India.

### References

1. Bird MI, Foster MR, Priest R, Malhotra R. Selectins: physiological and pathophysiological roles. *Biochem Soc Trans* 1997; 25 : 1199-206.
2. Dong ZM, Chapman SM, Brown AA, Frenette PS, Hynes RO, Wagn DD. The combined role of P- and E-selectins in atherosclerosis. *J Clin Invest* 1998; 102 : 145-52.
3. Poncz M, Solowiejczyk D, Harpel B, Mory Y, Schwartz E, Surrey S. Construction of human gene libraries from small amounts of peripheral blood: analysis of beta-like globin genes. *Hemoglobin* 1982, 6 : 27-36.
4. Ye SQ, Usher D, Virgil D, Zhang LQ, Yochim SE, Gupta R. A pstI polymorphism detects the mutation of serine128 to arginine in CD62E gene- a risk factor for coronary artery disease. *J Biomed Sci* 1999; 6 : 18-21.
5. Yoshida M, Takano Y, Sasaoka T, Izumi T, Kimura A. E-selectin polymorphism associated with myocardial infarction causes enhanced leukocyte-endothelial interactions under flow conditions. *Arterioscler Thromb Vasc Biol* 2003; 23 : 783-8.
6. Abu-Amero KK, Al-Boudari OM, Mohamed GH, Dzimiri N. E-selectin S128R polymorphism and severe coronary artery disease in Arabs. *BMC Med Genet* 2006; 7 : 52-5.
7. Miller MA, Kerry SM, Dong Y, Sagnella GA, Cook DG, Cappuccio FP. Circulating soluble E-selectin levels and the Ser 128Arg polymorphism in individuals from different ethnic groups. *Nutr Metab Cardiovasc Dis* 2005; 15 : 65-70.
8. Mohan V, Deepa R, Rani SS, Premalatha G; Chennai Urban Population Study. Prevalence of coronary artery disease and its relationship to lipids in a selected population in south India. *J Am Coll Cardiol* 2001; 38 : 682-7.

9. Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, Loscalzo J, eds. Harrison's Principles of Internal Medicine. 17<sup>th</sup>ed. New York: McGrawHill; 2008.
10. Li Y, Wei YS, Wang M, Zhang PA, Jiang XJ, Huang CX. Association between the Ser128Arg variant of the E-selectin and risk of coronary artery disease in the central China. *Int J Cardiol* 2005; *103* : 33-6.
11. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* 1982; *28* : 2077-80.
12. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; *20* : 470-5.
13. Lopes-Virella MF, Stone P, Ellis S, Colwell JA, *et al*. Cholesterol determination in high-density lipoprotein & separated by three different methods. *Clin Chem* 1977; *23* : 882-8.
14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; *18* : 499-502.
15. Mager A, Lalezari S, Shohat T, Birnbaum Y, Adler Y, Magal N, *et al*. Methylenetetrahydrofolate reductase genotypes and early-onset coronary artery disease. *Circulation* 1999; *100* : 2406-10.

*Reprint requests:* Dr Sarita Agarwal, Department of Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS)  
Lucknow 226 014, India  
e-mail: sarita@sippi.ac.in