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PDE4D Gene Polymorphisms and Coronary Heart Disease: A Case-Control Study in a North Indian Population

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Background: The present study aims to assess the association of PDE4D gene polymorphisms (SNP83 and SNP87) with Coronary Heart Disease (CHD) in a single Mendelian population of Delhi. *Methods:* A cross-sectional study was carried out wherein intravenous blood samples were collected from 100 cases and 100 age, sex and ethnicity matched controls along with their demographic, life style, and clinical profiles. *Results:* Genotypic frequencies of PDE4D gene variants 83 and 87 did not differ significantly between cases and controls. Odds ratio revealed a 1.4-fold increased risk with PDE4D 83 C allele; though not significant. Both the SNPs showed significant association with serum triglyceride (TG) ($P \leq 0.05$). A significant linkage disequilibrium was observed between the SNPs. The haplotype with mutant alleles of the two SNPs showed fivefold increased risk (though not significant) and that with normal allele of SNP 83 and mutant allele of SNP 87 (T-T) was found to be significantly associated with the disease in the present population. *Conclusions:* PDE4D gene variants 83 and 87 did not show any significant association with CHD. However, their interaction with TG and the haplotypic association found in the present population is indicative of the population-specific risk associated with CHD where majority of the individuals have high cholesterol and high Body Mass Index (BMI). J. Clin. Lab. Anal. 27:297–300, 2013.

Key words: Coronary Heart Disease (CHD); haplotype; North Indian Mendelian Population; PDE4D gene polymorphisms

Abbreviations

CHD = Coronary Heart Disease
PDE4D = phosphodiesterase 4D

The extent of polymorphism and the manifestation of candidate genes in various diseases vary among different ethnic groups as they are largely influenced by mating pattern, surrounding genetic environment, life style factors, and other environmental factors that are population specific. India with enormous diversity is expected to have unique gene pools, specific to communities that in turn are likely to be specific with respect to disease causation and expression. Since the initial identification of phosphodiesterase 4D (PDE4D) as a risk gene for ischemic stroke, there have been numerous replicative studies across the globe with conflicting results. The gene is widely expressed and regulates intracellular levels of cyclic-AMP (1). Atherosclerotic pathophysiology is responsible for the cause of stroke and Coronary Heart Disease (CHD), thus in the present study an attempt is made to analyze the association of PDE4D gene polymorphisms (83 and 87) and their haplotypes among atherosclerotic CHD patients from a single Mendelian population of Delhi (India). The population is an elite class exhibiting sedentary lifestyle and a fatty diet consumption and therefore, is likely to be at a high predisposition to CHD.

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The study was carried after the approval from the ethical committee of Department of Anthropology, University of Delhi and also Aggarwal Dharmarth Hospital. Blood samples from 100 cases (clinically diagnosed Angina and MI patients) and 100 controls matched on age, sex, ethnicity, geography, and food habits. Controls included the individuals who showed no symptoms of CHD, diabetes, hypertension, and family history of CHD. Demographic data pertaining to age, sex, etc. were taken with the help of interview schedule. Anthropometric measurements like height, weight, hip and waist circumferences were taken following the standard methods (2).

Intravenous blood samples were collected from the subjects with the understanding and written consent of each subject. 8 ml of fasting blood was collected from controls; of which 3 ml was used for lipid profile using commercial kit (Auto pack, diagnostic test kit for in vitro use, SIEMENS). For cases, lipid levels measured before surgery or the treatment were taken, so as to ensure that there is no effect of lipid lowering drug treatment. Remaining 5 ml of blood from controls and 5 ml fresh blood collected from cases were subjected to DNA extraction using salting out procedure (3). PCR amplification and restriction digestion were carried out following previously described protocols (4).

Genotype and allele frequencies were calculated by gene counting method. Hardy-Weinberg equilibrium was tested among cases and controls. Odds ratios were calculated along with 95% confidence interval to calculate the risk of each studied polymorphism associated with CHD. Multivariate logistic regression model was applied with adjustment for confounding variables like BMI, WHR, and lipids (SPSS 15.0 version). Haplotype frequency and linkage disequilibrium (LD) were calculated using SNPstats (5).

Cases and controls did not differ significantly with respect to lifestyle factors, anthropometric variables, and biochemical variables, except for triglyceride ($\chi^2 = 20.04$, df = 1, P value = 0.00) and low density lipoprotein ($\chi^2 = 24.30$, df = 1, P value = 0.00). The mean values of cholesterol, BMI, and WHR were found to be higher than the normal recommended range among both cases and controls (data not shown).

Of the 100 patients screened for the selected mutation of PDE4D gene both the SNP83 and SNP87 polymorphisms were found to be high in both heterozygous and mutant homozygous conditions among controls as compared to cases, although the difference was not found to be significant (Table 1).

When odds ratio was computed, individuals carrying mutant (C) allele of SNP 83 showed an increased risk with CHD; though not significant ($P = 0.200$) while those carrying mutant (T) allele of SNP 87 did not show any increased risk. The multivariate logistic regression analysis after controlling the various confounding variables revealed no significant risk associated with CHD for both the PDE4D gene variants.

On testing the difference between means of various dependent biochemical and anthropometric variables for SNP 83 and SNP 87 the one-way ANOVA and Kruskal–Wallis test showed no significant difference in the mean value of all the three genotypes except that for serum triglyceride for SNP 87 (0.012 and 0.017, respectively).

The two SNPs were found to be in strong linkage disequlibrium ($D' = 0.646$; $P = 0.004$). Haplotype analysis revealed that the frequency of T-C (wild type) haplotype was found to be high among cases (59.98%) and the frequency of C-T (mutated type) haplotype was found to be high among controls (3.9%) as compared to cases (3.17%). The haplotype with mutant alleles of both the SNPs showed approximately sixfold risk associated with CHD; though not significant (OR: 5.89; CI 95: 0.73–47.44; $P = 0.098$), while the haplotypic combination of normal allele of SNP 83 and mutant allele of SNP 87 was found to show 11-fold significant risk associated with CHD (OR: 11.06; CI 95: 10.99–11.13; $P < 0.0001$).

Studies have been conducted on association of PDE4D 83 and 87 gene variants with ischemic stroke (6–9) revealing conflicting results. In a meta analysis on association between variation in PDE4D gene and ischemic infarction risk in Asian populations (10), the authors pooled all the studies conducted from January 1984 to June 2009 that included five Asian population groups (Chinese, Pakistani, Indian, Japanese and Korean). These studies included various subtypes of Ischemic stroke (embolic, nonembolic, cardiogenic, noncardiogenic, atherothrombotic stroke). In this meta-analysis, PDE4D was found to be associated with ischemic infarction in Asian people, and SNP 83 was found to be an important biomarker. Independent studies on nonembolic (thrombotic and lacunar) stroke (11) and cardiogenic and carotid ischemic stroke (12), respectively, revealed a positive association of SNP 83 and 87. PDE4D encodes c-AMP specific 30, 50-cyclic phosphodiesterase 4D that is a member of the phosphodiesterase super family (PDE4 family) expressed in most cell types that are involved in the pathogenesis of atherosclerosis(13). Atherosclerosis is a causative factor underlying the pathogenesis of cardiovascular diseases (CVD) including both CHD and stroke. Therefore, the role of this gene should be investigated in causation of not only stroke but also of CHD. Present study is first of its kind in Indian context to assess the role of PDE4D gene variants (83 and 87) in the pathogenesis of CHD.

In the present study, genotypic and allelic distributions of the two SNPs revealed no significant differences among cases and controls. However, although not significant, 1.4-fold increased risk could be observed for the association

TABLE 1. Distribution of Gene and Genotypic Frequencies of PDE4D Gene Polymorphism (SNP 83 and SNP 87) Among Cases and Controls and Risk Calculation

Loci at PDE4D	Genotype	Cases ($N = 91$)[a]	Controls ($N = 95$)[a]	P value[b]	OR (95%CI)[c]	P-value[d]
SNP83	TT	35 (38.89%)	35 (36.84%)	0.95	1	
	TC	39 (43.33%)	42 (44.21%)		0.929 (0.489–1.761)	0.82
	CC	16 (17.78%)	18 (18.95%)		0.889 (0.391–2.019)	0.78
	TC + CC	55 (61.11%)	60 (63.16%)		1.441 (0.823–2.522)	0.20
	T allele	0.60	0.58	0.75		
	C allele	0.40	0.42			
		$N = 93$[a]	$N = 84$[a]			
SNP 87	CC	88 (94.62%)	77 (91.67%)	0.19	1	
	CT	3 (3.23%)	4 (4.76%)		0.656 (0.14–3.024)	0.71
	TT	2 (2.15%)	3 (3.57%)		0.583 (0.095–3.58)	0.44
	CT + TT	5 (5.38%)	7 (8.33%)		0.625 (0.191–2.049)	0.43
	C allele	0.96	0.84	0.34		
	T allele	0.04	0.16			

[a]Results from PCR analysis were not obtainable from the remaining DNA sample.
[b]Calculated through χ^2-test.
[c]Odds Ratio (OR) calculated at 95% confidence interval (CI).
[d]Calculated for odds ratio.

of SNP 83 with CHD and significant interaction was observed with high TG (hypertriglyceridemia), which is an established risk factor for CHD. SNP 87 did not show any increased risk with CHD but was significantly associated with high TG. Significant interaction of the two SNPs with high TG directs toward the risk of these polymorphisms in individuals with hypertriglyceridemia. Moreover, CHD is a multifactorial disorder wherein both genetic and environmental factors play an important role in the pathogenesis of the complex disease. Therefore, gene–environment interaction in the pathophysiology of the complex diseases should be given prime attention. Present study sheds light on gene–environment interaction of PDE4D gene in relation to CHD. High frequency of T-T haplotype among controls and its association with the disease is in concordance with the Icelandic population (14) where the selective disadvantage of this haplotype was observed in the population. Also, SNP 83 and SNP 87 that fall in the same block structure of the gene as described in the Icelandic population (14) tend to stay together. Hence, the significant LD between the two SNPs hints toward the additive effect of the haplotypes rather than the individual SNP as such. Small sample size is limitation of the study but strength includes the homogeneity with respect to selection of cases and controls that are from a single North Indian Mendelian population and are hence, matched on ethnicity, geography and food habits along with age and sex.

Conclusively, the PDE4D gene variants (83 and 87) may not be directly associated with CHD, but their significant interaction with serum triglyceride (an important lipomarker for CHD) is suggestive of their indirect increased risk for CHD in individuals with hypertriglyceridemia. Moreover, their haplotypic association and strong LD hint toward the population specific risk associated with CHD. PDE4D gene variants 83 and 87, thus, may not be causally associated with CHD, but rather may indirectly associate with risk agents of CHD like hypertriglyceridemia and also may be in linkage disequilibrium with each other (which may differ across populations), such as found in present population where majority of the individuals have high cholesterol and high BMI. Further studies with larger sample size should be conducted to identify causative genetic variants in PDE4D gene and their susceptibility for CHD keeping ethnic variations under their purview.

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