9p21.3 Locus is Associated in The Risk of Coronary Artery Disease in Tanzanian Patients

Gokce Akan[1], Peter Kisenge[2], Tulizom Shemu Sanga[2], Erasto Mbugi[1], Mohammed Janabi[2] and Fatmahan Atalar[1,3]

[1]Biochemistry Department, School of Medicine, MUHAS, Dar es Salaam-Tanzania
[2]Jakaya Kikwete Cardiac Institute (JKCI), Dar es Salaam-Tanzania
[3]Istanbul University, Child Health Institute, Medical Genetics Department, Istanbul -Turkey

Background: Coronary Artery Disease (CAD) is the most common heart disease. CAD is the result of plaque buildup in the coronary arteries that leads to blockage. Lifestyle and environmental factors play an important role in their development, but genetic inheritance appears to be strongly involved. Recently, genome-wide association studies (GWAS) have revealed single nucleotide polymorphisms (SNP) on chromosome 9p21.3 that confer susceptibility to CAD. The 9p21.3 risk locus spans a >50-kb genomic region and comprises 59 linked SNPs and association of 9p21.3 risk variants with CAD has been confirmed with replication in multiple white cohorts and populations of different ethnicities (Figure 1). But this association is not documented in Tanzanian cohorts.

Aim: The aim of the study is to investigate the common SNPs rs1333049, rs2383207, rs2383206, rs10757274, rs10757278 and rs10811656 in 9p21.3 in Tanzanian CAD patients and their associations with severity of CAD, demographical and biochemical parameters.

Materials & Methods: 135 patients with CAD (age 62.01±10.65) and 140 non-CAD (age 58.21±12.62) patients were enrolled into the study. Further the biochemical tests, the SNP analysis were performed by the use of genomic DNA obtained from 400ul peripheral blood leukocytes with MagnaPure Compact and DNA quantity was determined by NanoDrop Spectrophotometer. Genotyping of the SNPs by performed with the LightSNIP typing assay using a Quantitative Real-Time PCR (QRT-PCR) method. The experiment results were examined in Melting Curve analysis program (Figure 2).

Results: Our analyses revealed that clinical and biochemical parameters (BMI, Glucose, Cholesterol, HDL, LDL, VLDL, TG, ALT and AST levels) were significantly different between the CAD and non-CAD patients (Table 1) ($p<0.05$, respectively). The genotype distributions of rs1333049C/G, rs2383207A/G, rs2383206A/G, rs10757278A/G, rs10757278G/A, rs10811656C/T were significantly different between the groups ($p<0.05$, respectively) (Figure 3). Subgroup analysis of CAD patients revealed a significant interaction of the risk genotypes of rs10757274 and rs10757278 with hypertension in conferring increased risk of CAD. Although the differences were observed, no statistical significance was reached in diabetes and obese subgroups (Table 2).The genotype distribution of rs1333049, rs10757278 and rs10811656 polymorphisms were significantly different among patients with one, two, three stenotic vessels ($p<0.05$) (Table 3). The analysis of the six-SNP haplotypes displayed significant differences in the distributions of three haplotypes, additionally, haplotype analysis revealed that AAGCAG, AAACAG, GGGTGC haplotypes of 9p21.3 locus polymorphisms are associates with CAD risk (Figure 4). The GGGTGT haplotype was overrepresented while the other two underrepresented in patients as compared to controls ($p<0.00001$) suggesting the first one a high risk and the other two low-risk haplotypes for Tanzanian population. Moreover, glucose, cholesterol, HDL, LDL levels were statistically significant in rs10811656 CT and TT female carriers compared to CC (WildType) female carriers ($p<0.05$, respectively).

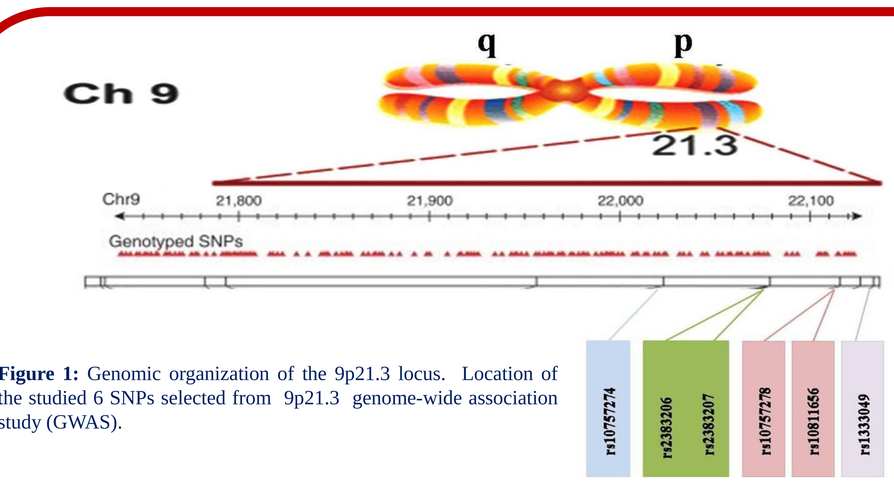


Figure 1: Genomic organization of the 9p21.3 locus. Location of the studied 6 SNPs selected from 9p21.3 genome-wide association study (GWAS).

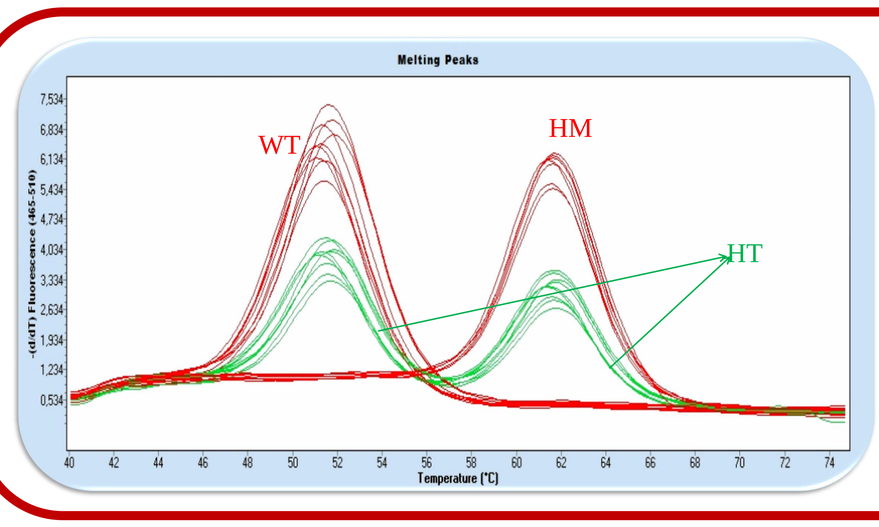


Figure 2: Genotyping result of the SNPs examined in Melting Curve analysis program.

WT: Wild Type,
HM: Homozygous Mutant
HT: Heterozygous Mutant

Table 1: Clinical Characteristics of study group

Variables	CAD patients (n=135)	Non-CAD patients (n=140)	P Value*
Age (years)	62.01±10.65	58.21±12.62	0.08
BMI (kg/m2)	34.78±4.43	26.18±3.72	*0.000*
Glucose (mmol/L)	7.44±2.75	3.92±1.03	*0.000*
Cholesterol(mmol/L)	6.16±1.03	4.09±0.87	*0.000*
HDL (mmol/L)	0.90±0.25	1.34±0.30	*0.000*
LDL (mmol/L)	4.06±0.96	2.68±0.69	*0.000*
VLDL (mmol/L)	1.20±0.46	0.36±0.19	*0.000*
TG (mmol/L)	2.35±1.48	1.10±0.48	*0.000*
ALT (U/L)	31,97±16.66	19.6±7.11	*0.000*
AST (U/L)	42.07±4.95	22.54±6.78	*0.002*

Data: mean ± SD*Comparisons of differences between mean values of two groups unpaired Student t-test was used. In all cases differences were considered significant at $p< 0.05$.

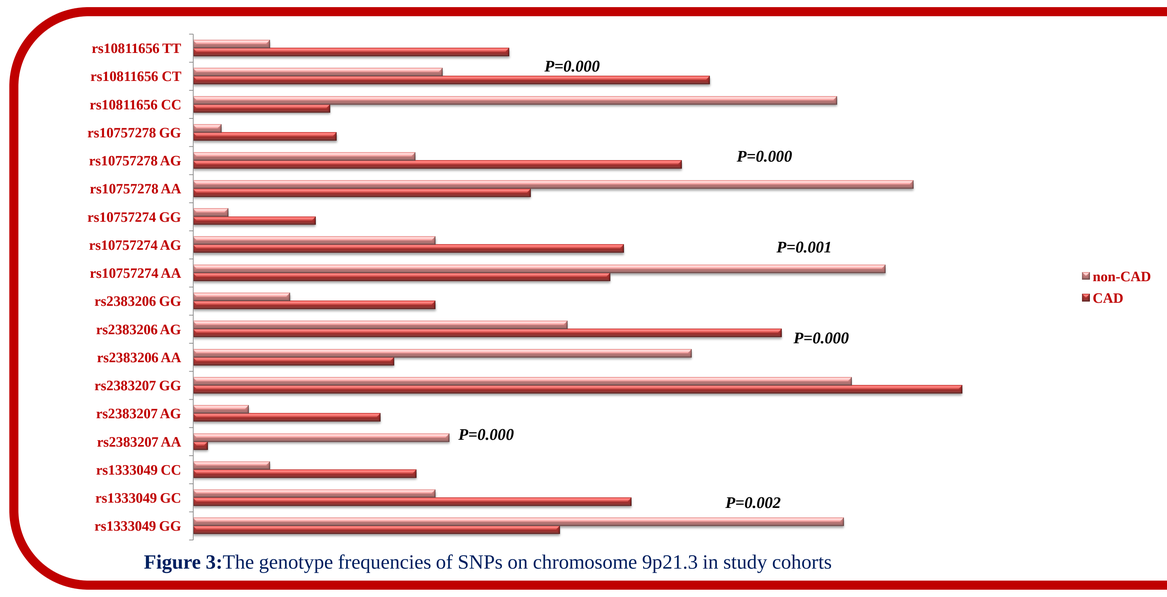


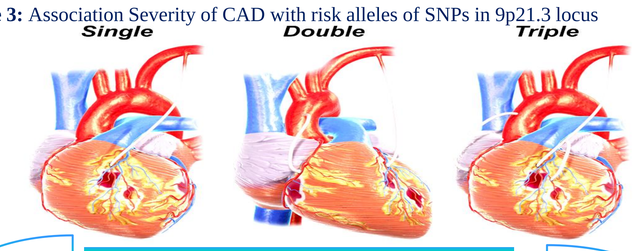
Figure 3: The genotype frequencies of SNPs on chromosome 9p21.3 in study cohorts

Table 2: SNP analysis of CAD in hypertensive subpopulation

SNP rs10757274	GA+GG		AA		OR*	95%CI	P-Value
.	CAD(n=135)	non-CAD(n=140)	CAD(n=135)	non-CAD(n=140)			
Hypertension							
Yes	73	38	56	98	3.36	2.01-5.6	*0.002*
No	4	2	2	2	2.00	0.15-2.67	0.59
SNP rs10757278	**GA+GG**		**AA**		**OR***	**95%CI**	**P-Value**
	CAD(n=135)	non-CAD(n=140)	CAD(n=135)	non-CAD(n=140)			
Hypertension							
Yes	85	34	44	102	5.79	3.40-9.86	*0.000*
No	3	2	3	2	1.00	0.06-2.66	0.85

OR: Odd Ratio *The allelic frequency distribution of polymorphisms between the groups was compared using Hardy Weinberg Equilibrium (HWE) test. In all cases differences were considered significant at $p< 0.05$.

Table 3: Association Severity of CAD with risk alleles of SNPs in 9p21.3 locus



	Single Vessel(n=16)	Double Vessels(n=23)	Triple Vessels(n=15)	
SNP	Ref.	P-Value	OR	95% CI
rs1333049	G	*0.015*	0.739	0.580-0.942
rs2383206	A	0.380	2.294	0.396-1.32
rs2383207	A	0.483	4.940	0.530-4.604
rs10757274	A	0.226	0.840	0.502-6.097
rs10757278	A	*0.005*	2.100	0.197-2.23
rs10811656	C	*0.015*	0.760	0.627-4.82

OR: Odd Ratio, CI: Confidence Interval *The allelic frequency distribution of polymorphisms between the groups was compared using Chi square (x2) test. In all cases differences were considered significant at $p< 0.05$.

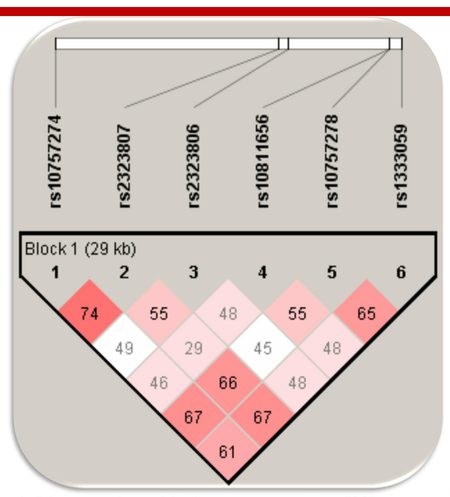


Figure 4: Linkage disequilibrium pattern of the SNPs along the 9p21.3 region

Conclusion: Our results have shown that; **1-** six common genetic variants within the chromosome 9p21.3 region are associated with CAD in Tanzanian patients. **2-**rs1333049, rs10756278, and rs10811656 were found to increased the severity of CAD in Tanzanian patients **3-** rs10811656 plays a major role in a gender-specific manner which would trigger the CAD development in Tanzanian females. **4-** Allele distributions and genotypes of 9p21.3 region SNPs are significantly associated with CAD risk in Tanzania and further confirm the very first haplotype block; GGGTGC, harboring the disease-causing SNPs in Eastern African Tanzanians.