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Original Article

Association of SNP rs2487928 on chromosome 10p11.2 with the risk of coronary artery disease based myocardial infarction in a Saudi population

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Abstract: Coronary artery disease (CAD) is one of the most common causes of morbidity and mortality worldwide. Genome wide association studies (GWAS) on Caucasian populations have revealed a major risk locus for CAD on chromosome 10p11.2. Therefore, we conducted an association study of the three most common SNPs reported on this locus (rs 2487928 A/G, rs2505083 C/T, rs 3739998 C/G) in a selected case control model to shed some light on the genetic factors associated with the occurrence of CAD in the Saudi population. A total of 1,004 chromosomes (500 chromosomes from Saudi CAD patients, who had experienced at least one myocardial infarction (MI) event and 504 chromosomes from Saudi age-matched healthy controls) were genotyped using real time PCR based TaqMan assay. Linkage disequilibrium (LD) analysis of three SNPs using haploview showed a significant difference in the genotype distribution for the SNP rs 2487928G between patients and controls (P = 0.00840, OR 1.4161, 95% CI 1.0930-1.8348, χ^2 6.95). The other two SNPs, rs 3739998 and rs 2505083, were found to be CAD protective. Quality control of TaqMan results was carried out on 5% of the samples using Dye-terminator sequencing, the results of which concurred with the primary TaqMan genotypes. This study is in line with other studies that reported that the SNP rs2487928 G/G genotype located in KIAA1462 gene at 10p11.2 locus is significantly associated with CAD in other populations.

Keywords: Myocardial infarction, coronary artery disease, single nucleotide polymorphism, Saudi Arabia, risk allele, haploview, DNA sequencing

Introduction

Coronary Artery Disease (CAD) based myocardial infarction (MI) is one of the major causes of morbidity and mortality worldwide and places a significant socio and economic burden on society [1, 2]. Cardiovascular diseases are prevalent in Saudi Arabia, with the Eastern Province, where the study subjects originated, having one of the highest incidences. Approximately 60% of sudden death incidences in the Eastern Province are caused by CAD [3-9].

Even though numerous risk factors, including obesity, diet, and smoking, are involved in the development of CAD, the genetic basis of CAD remains unclear. Genome wide association studies (GWAS) have identified the locus 10p11.2, as increasing the susceptibility toward the development of CAD [10]. Several studies have also revealed a significant association of specific SNPs (rs 3739998, rs 2505083, rs 2487928) in the 10p11.2 loci with CAD [10-14]. Recent reports have emphasised that the occurrence of CAD in the Saudi population is increasing especially in young adults [5, 7-9, 15].

Understanding the genetic basis of CAD may contribute towards identifying better targets for innovative CAD drugs. As far as the authors are aware, there are no reports on the genetic variants on 10p11.2 loci in the development of CAD in an Arab population. Therefore, we tested the association of risk variants at three SNPs on 10p11.2 locus with the development of CAD

Table 1. Clinical features of individuals with CAD and controls without CAD

Features*	Case (n = 250)	Controls (n = 252)
Gender, M/F	163/87	200/52
Age (Y)	52.3±13.8	48.07±7.9
BMI Kg/m^2	35.59±5.3	27.90±3.03
Hypertension (%)	69.6	7.41
Diabetes (%)	59.2	4.81

*Data are shown as percentage or mean ± standard deviation.

based MI in the Saudi Arabian population in a case control model.

Materials and methods

Study subjects

The study population included 250 Saudi CAD patients who had experienced at least one MI, and had been admitted to one of the major hospitals in Al-Ahsa, Qatif and Al-Khobar in the Eastern Province of Saudi Arabia. A total of 252 aged matched healthy volunteers, attending the blood bank of the same hospitals, were randomly selected for the control group. The study was approved by the Institutional Review Board at the University of Dammam. After obtaining written informed consent from all volunteers, 5ml of whole blood was collected from each volunteer. Clinical characterization of all patients is shown in **Table 1**.

TaqMan® SNP genotyping

Genomic DNA was extracted from 150 µl whole blood using Wizard Genomic DNA purification kit, Promega, USA from blood samples of patients and controls. TaqMan® assay (Applied Biosystems, CA, USA) based SNP genotyping was carried out using the Rotor-Gene Q (Qiagen, USA) real time PCR System. Genotyping of three SNPs (rs 3739998, rs 2505083, rs 2487928) was carried out separately on 20 ng of genomic DNA in a 15 µL volume with 2 × TaqMan master mix. Rotor-Gene Q Series Software 2.0.2 (Build 4) (Corbett Life Science, USA) was used to identify the alleles. Furthermore, the DNA samples for controls and cases were genotyped in the same batches.

Sanger sequencing - quality for SNP genotyping

Primers were designed for the amplification of ~150 bp up and downstream to the SNP position (**Table 3**) and amplified separately using the PCR (BIO-RAD MyCycler™). PCR solution included top *Taq* buffer (10 ×) (5 µl), 100 ng of DNA, 25 mM dNTP (0.4 µl), 25 mM $MgCl_2$ (3 µl), 10 µM forward and reverse (respective primers accordingly) oligos (2 µl), top *Taq* DNA polymerase 5 U/µl (0.2 µl) and water (to 50 µl). Cycling temperature was 95°C/5 minutes, 30 PCR cycles of 30 sec at 95°C; 30 sec at 56°C, and 30 sec at 72°C, final extension at 72°C/7 min. The amplified products were purified (QIAquick PCR Purification Kit, Qiagen, Germany) and subjected for cycle sequencing with a total volume of 20 µL (Cycle sequencing kit, Qiagen, Germany). Cycling conditions: 1 min at 96°C; 25 cycles of 10 sec at 96°C; 5 sec at 50°C; 4 min at 60°C and stored at 4°C. Cycle sequenced products were purified and were separated using POP 7 in a 3500 Genetic Analyzer (Applied Biosystems, USA). Sequencing Analysis Software V5.4 (Applied Biosystems, CA, USA) and MAFFT version 7 were used to quality the genotypes obtained using TaqMan® assay.

Statistical analysis

Genotyping data from the 252 control subjects for the three SNPs (10p11.2 loci) were subjected to the Hardy-Weinberg equilibrium test to confirm independent segregation of the alleles using haploview version 4.2. [16]. A statistical power analysis was implemented using a case-control design. Allelic association of a SNP on cases and controls was measured using χ^2 test using haploview. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using web based MedCalc®. Risk alleles were identified using haploview. Non-random association of 3 alleles at 10p11.2 were tested by haploview based linkage disequilibrium (LD). Statistical significance was maintained at $P \leq 0.05$. Haplotype blocks were constructed from genotyping data using haploview.

Results

A total of 250 Saudi CAD patients who had experienced at least one MI event and 252 Saudi age matched healthy controls were included in the study (**Table 1**). The cases and controls delivered 90% of statistical power at the Type I error rate of 0.05. The three tested SNPs, rs 3739998, rs 2505083 and rs 2487928, showed no significant deviation ($P > 0.05$) from the Hardy-Weinberg equilibrium

Table 2. Association of CAD with risk alleles of SNPs in 10p11.2 locus

SNP ID	Associate Allele	P-HW	ObsHET	PredHET	Odds ratio (95% CI)	Case; Control ratio	χ^2	*P* value
Rs 3739998	G	0.4936	0.47	0.487	1.2872 (0.9906-1.6725)	206:252, 181:285	3.574	0.05870
Rs 2505083	T	0.0415	0.434	0.48	1.0550 (0.8152-1.3653)	301:195, 278:190	0.165	0.68420
Rs 2487928	G	0.7785	0.475	0.483	1.4161 (1.0930-1.8348)	313:181, 254:208	6.95	0.00840*

P-HW, *P* value for Hardy-Weinberg equilibrium analysis; Obs (HET): Observed heterozygosity; Pred (HET): Expected or predicted heterozygosity; *Significant at $P < 0.05$.

Table 3. List of primers designed for the identification of 6 SNPs through Sanger Sequencing

Name of the SNP	Primer name*	Target specific sequence (5' to 3')	Melting at (°C)	Product size (bp)
Rs 3739998	9998F	GTGCTTGCTTCACCCAAAGACCCCTCT	68.77	361
	9998R	GTGCAGAGAAGAGACACCTGGAGGTTAGC	68.01	
Rs 2487928	7928F	TGAGAAATGCTGTAGAACATGAACCA	60.97	416
	7928R	TGTACTTGCACAAGTAGCACCT	59.90	
Rs 2505083	5083F	CTTAAAGAAAGGTCCCTGATTCCGT	60.80	400
	5083R	ATCATTCCATTGGCTTCTGACCTATC	60.97	

*Forward primers (F) of the respective products were used as sequencing primer.

(HWE) test in the control group (**Table 2**). Linkage disequilibrium (LD) analysis of SNPs indicated a significant difference in the genotype distribution for one SNP, rs 2487928, between the patients and controls (P = 0.00840; *OR* 1.4161, 95% CI 1.0930-1.8348, χ^2 6.95). The other two SNPs did not show any association with CAD.

LD plot was constructed based on the pairwise correlation between the three SNPs on chromosome 10p11.2 locus to identify the linked variants. The measured r^2 revealed notable CAD protective variants (GT: rs 3739998 and rs 2505083) (control r^2 = 0.44; cases r^2 = 0.00) were found to be recessive (**Figure 1**).

The quality of the results using TaqMan® assay was confirmed by direct DNA sequence analysis of 5% of the samples using newly designed primers (**Table 3**), the results of which were in agreement with the initial results (**Figure 1**). To determine whether the two protective loci (rs 3739998 and rs 2505083) were independent of one another, we conducted a LD test. The results (D' = 0.39) showed that the alleles of rs3739998G and rs2505083T are 39% surrogate for each other in the study cohort (**Figure 1**).

Discussion

The prevalence of obesity, cardiovascular diseases and diabetes has been increasing in Saudi Arabia during the last five decades due to socio-economic changes [2, 17]. However, the role of genetic factors that influence the development of CAD cannot be overlooked. Genetic variants play a significant role in conjunction with other known traditional risk factors for the development of CAD [18]. A meta-analysis study of CAD, including a number of GWAS, identified 46 genetic loci that show an association with CAD. One of these loci which consistently shows a strong association in many populations which was identified on chromosome 9p21 [10-14]. Another genetic locus identified on chromosome 10q11.2 has also shown a considerable association with CAD [19]. Within this locus, three SNPs (rs 3739998, rs 2505083, rs 2487928) were identified which showed a significant association with CAD in both European and Asian populations [10-14]. The present study investigated whether there was an association between these three SNPs on chromosome 10q11.2 and the increased risk of the development of CAD in the Saudi population of the Eastern Province.

The Eastern Province of Saudi Arabia has been reported to have one of the highest incidences of CAD in the Kingdom [7]. A recent report has also shown that approximately 60% of sudden deaths in the Eastern Province are as a direct result of CAD [7]. When determining the criteria for the study group, we selected cases and controls over the age of 45 years due to the high

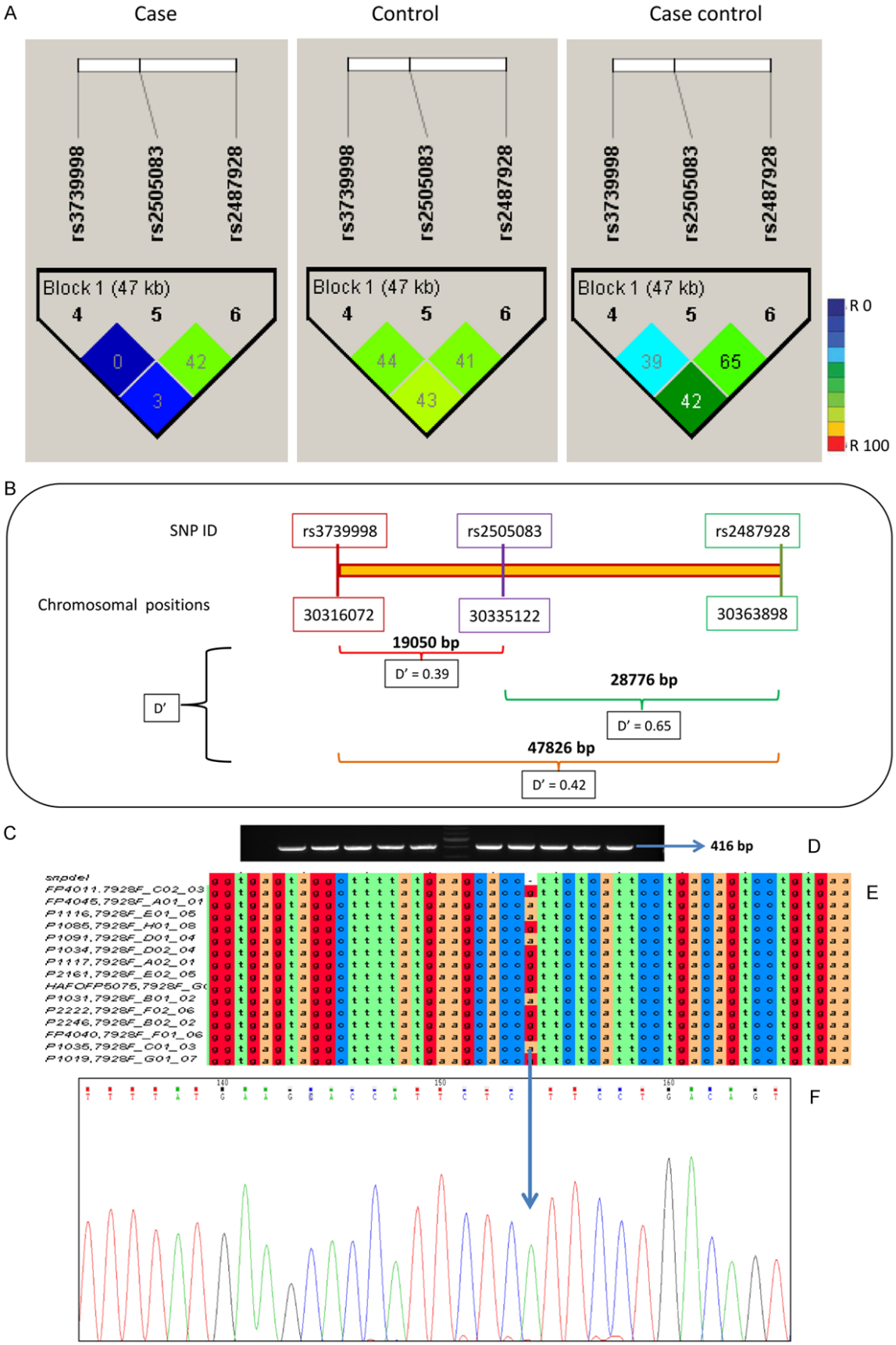
A
Case
Control
Case control
rs3739998
rs2505083
rs2487928
Block 1 (47 kb)
4
5
6
0
42
3
44
41
43
39
65
42
R 0
R 100
B
SNP ID
rs3739998
rs2505083
rs2487928
Chromosomal positions
30316072
30335122
30363898
19050 bp
D' = 0.39
28776 bp
D' = 0.65
47826 bp
D' = 0.42
D'
C
416 bp
D
E
F

Figure 1. A. Haploview LD (linkage disequilibirum) plot of the SNPs in 10p11.2 locus. The pairwise correlation between the SNPs were measured as r^2 and shown (x 100) in each diamond. B. The chromosomal positions of the 6 SNPs analyzed in 10p11.2 and their linkage disequilibrium (LD). Lower half: Indicating the D' value among 3 SNPs in 10p11.2. Coordinates are according to the reference sequence NT_008413.18. C. DNA sequence chromatogram of rs2487928 D. Amplified product in 1% agarose gel, middle lane: 100 bp ladder; both the side of ladder: 416 bp amplicon. E. MAFFT alignment. F. Electropherogram.

incidence rate (11.7%) of CAD in that age group in the Saudi population [3-6].

This study is the first to report an association between rs2487928 on chromosome 10p11.2 and CAD in an Arab population. Our findings are in line with previous findings on other population [10]. The other two SNPs, rs 3739998 and rs 2505083, have been shown to have a significant association with CAD in other populations, but there was no statistically significant association in the present population for these two SNPs [10-14].

It has to be noted that consanguineous marriages and genetic variations are quite common in Saudi Arabia [20-25]. This is probably reflected in the increased prevalence of these polymorphisms among the population. Consequently, in addition to other factors, this might lead to an increased prevalence of CAD.

Conclusion

Statistical evidence (LD analysis: *D'* and r^2 and genotypic association analysis) revealed that the SNP rs2487928 is associated with the development of CAD in the Saudi population (p = 0.00840, χ^2 6.95). While linked variant in 10p11.2 locus, GT: rs 3739998 and rs 2505083 were found to be CAD protective (control r^2 = 0.44; cases r^2 = 0.00) and are 39% surrogate for each other for each other in the study cohort.

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Disclosure of conflict of interest

None.

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