"Desert" gene (Chr9p21) variants as novel markers for coronary artery disease

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Abstract

Objective: Previous reports have denoted to the possible link of Chr9p21 locus to the incidence of coronary artery disease (CAD). The entire core of chr9p21 is covered by "ANRIL" (Antisense noncoding RNA in INK4 Locus) and lies in a region that is free from any coding proteins; therefore, it is called the desert gene. The major objectives of this study were to examine the association of rs10757278 and rs2383206 SNPs on Chr9p21 with the incidence of CAD in the presence and absence of type 2 diabetes (T2D) in Egyptians and to correlate these genetic variants with several disease biomarkers (TC, CRP, and HbA1c).

Methods: The study subjects consisted of 150 subjects; 50 healthy controls and 100 patients that were divided into two groups; CAD patients and CAD T2D patients. The genotyping of SNPs was performed using qPCR.

Results: Genotype distribution for both SNPs were found to be significantly different (p=0.0009 for rs10757278 and p=0.001 for rs2383206) between patients and controls. The allele frequency was also different for rs10757278.

Conclusion: The current study showed that rs10757278/rs2383206-G allele increases the risk for CAD in Egyptians. Moreover, AA variant appeared as a protective genotype. However, SNPs did not noticeably contribute in the elevation of TC, hs-CRP, and HbA1c in non-diabetic and diabetic CAD patients. *(Anatol J Cardiol 2017; 18: 84-9)*

Keywords: Desert gene (Chr9p21) polymorphism, Egyptians, coronary artery disease

Introduction

Chromosome 9 has around 1149 genes and 426 pseudogenes (1). Many of these genes are implicated in neurodegenerative diseases, male-to-female sex reversal, and cancer (1, 2). Among the loci on chromosome 9, chr9p21 [chromosome 9, short arm (P) at position 21] represents a fascinating spot to many scientists for several reasons; first, it comprises numerous genes that are correlated with various types of diseases such as type 2 diabetes (T2D) (3), glaucoma (4), endometriosis (5), Alzheimer disease (6), and periodontitis (7). As a matter of fact, chromosome 9p21 locus is one of the most common regions of deletions in cancer genetics that cause cutaneous malignant melanoma (8). It is also associated with other different types of cancer such as leukemia (9), breast cancer (10), pancreatic carcinoma (11), and ovarian cancer (12). Second, the haplotype core of chr9p21 is considered to be the most relevant associated signal for CAD (13, 14). Along with diabetes-associated hot spot, this segment refers to a region that is totally free from any coding proteins; thus, it is also known as "the desert gene" (15). Gene deserts are regions of the genome that are devoid of protein-coding genes and constitute around 25% of the entire genome. 9p21 desert region overlaps the sequence of a newly discovered long noncoding RNA [antisense noncoding RNA in INK4 Locus (ANRIL)]. Studies reported that ANRIL expression was clearly shown in vascular tissues and cells such as smooth muscle cells and monocytes that are relevant to atherosclerosis (14). Studies observed that SNPs that are responsible for changing the expression of ANRIL are associated with several diseases such as coronary artery disease (CAD), diabetes, and various cancers (16).

The third reason for making the chr9p21 locus more intriguing is the fact that genome-wide association studies pointed out to two loci for T2D mellitus that triggers the same region on chr9p21. What makes it a catch is the absence of any overlapping in the association signal between both loci, although these two loci lie on either side of the CAD-associated segment (14, 17).

Many SNPs on chromosome 9p21 have been associated with several diseases including cardiovascular disease (CVD). The most highly replicated associations to heart disease have been linked to SNPs in the haplotype core of chromosome 9p21 region

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Accepted Date: 07.04.2017 **Available Online Date:** 30.05.2017

DOI:10.14744/AnatolJCardiol.2017.7730

including rs2383206 and rs10757278, the two SNPs of interest in this study. Therefore, the major aims of this study were to examine the association of rs10757278 and rs2383206 SNPs on Chr9p21 with the incidence of CAD in the presence and absence of T2D in Egyptians and to correlate these genetic variants with several disease biomarkers. Total cholesterol (TC), high -sensitivity C-reactive protein (hs-CRP), and glycated hemoglobin (HbA1c).

Methods

Study population

Random unrelated 150 subjects were tested in this case-control study; 100 acute myocardial infarction patients, aged between 20 and 65 years and equally divided into type 2 diabetic and non-diabetic groups, were recruited from in- and out-patient settings. Fifty random unrelated age- and sex-matched non-diabetic controls with no diagnostic signs of CAD were also recruited for the study.

Patients were included if they had a diagnosis of single- or multi-vessel CAD as confirmed by coronary angiography, ECG changes, and/or elevation of cardiac markers. Written informed consent was obtained from each subject in this study that abided by the Helsinki Declaration. Information on personal and family medical histories and health-relevant behaviors, including diet and exercise, was obtained using a routine questionnaire filled in by blood donors at the time of venesection. Exclusion criteria for both patients and controls included any concomitant acute or chronic severe disease such as renal failure, hepatic insufficiency, and a CVD other than myocardial infarction (MI). Both patients and controls had a controlled blood pressure of below 140/90 mm Hg. The study protocol was approved by the Local Ethics Committees.

Specimen collection

Blood samples were collected from recruited subjects in vacutainers, which were centrifuged at 5000 rpm for 15 min at 25°C. The separated plasma and serum were stored at –80°C in a freezer, until use in the biomarkers analysis. Whole blood was used to measure HbA1c levels and for the genotyping analysis.

Genotyping

Genomic DNA was extracted from whole blood samples using Thermo Scientific Gene Jet whole blood genomic DNA purification minikit (Waltham, MA, USA, #k0781, lot number 00135002) following the manufacturer's instructions. The purified DNA was free of protein, nucleases, and other contaminants or inhibitors. DNA purity and concentration was determined using the NanoDropND-1000 spectrophotometer (Thermo, Wilmington, DE, USA) that measures 1-µl samples with high accuracy and reproducibility.

Determination of the rs10757278 and rs2383206 polymorphisms in the chromosome 9p21 locus was performed with the Applied Biosystems Real-Time PCR System (Stepone) (Foster City, CA, USA) using fluorescent-based PCR and Sequence Detection Systems software version 2.3 (Table 1).

Biochemical measurements

Serum TC and blood HbA1c levels were measured colorimetrically (Stanbio, Texas, USA), whereas hs-CRP was measured using ELISA technique (DRG instruments GmbH, Marburg, Germany EIA-3954).

Data processing and statistical analysis

Analyses were performed using GraphPad Prism statistics software (GraphPad Software, Inc., La Jolla, CA). Data are presented as mean±SEM of each parameter investigated. The association of the three different study groups with the three biomarkers (TC, hs-CRP, and HbA1c) was tested using non-parametric one-way ANOVA (Kruskal–Wallis test), followed by Dunn's multiple post-test to compare between each two groups. Association between the investigated polymorphisms and CAD was based on both genotypic and allelic models as shown by the χ^2 test. Odds ratios and the 95% CI were also calculated using the χ^2 test. Association of different genotypes of a SNP with each biomarker was tested using Kruskal–Wallis one-way ANOVA. The Hardy–Weinberg equilibrium and the linkage disequilibrium (LD) were both calculated using online calculators. Statistical significance was defined as a p value of ≤ 0.05.

Results

Characteristics of the studied groups

The general baseline characteristics of the studied groups participating in the study are shown in Table 2.

Genotyping of SNPs

rs10757278

The observed genotypes and alleles were found to be significantly different. It was noticeable that the difference in genotypes was remarkably significant (p=0.0009) (Table 3). Comparing the individual groups (CAD and CAD T2D) with controls yielded significant differences for CAD vs. control genotypes GG (p=0.001), AG (p=0.002), and AA (p=0.0002) with odds ratio (95% confidence intervals) for GG vs. AA, 3.55 (1.6–7.88) and for AG vs. AA, 3.92 (1.86–8.27) and for alleles (p=0.02) with odds ratio (95% confidence intervals) 1.91 (1.09–3.35).

For CAD T2D vs. controls, the p value for GG was 0.03, AG was 0.007, and AA was 0.089 with odds ratio (95% confidence intervals) for GG vs. AG, 2.66 (1.29–5.46) and for AG vs. AA, 1.82 (0.91–3.66), and for alleles it was 0.034 with odds ratio (95% confidence intervals) 1.831 (1.045–3.21).

In contrast, no significant difference was found for any of data upon comparing the genotype or allele distributions of CAD patients with those of CAD T2D patients.

rs2383206

Similar to the SNP rs10757278, the genetic variations of rs2383206 among studied groups were found to be highly significant (p=0.001). Meanwhile, the allele variations did not show enough significance (p=0.15) (Table 3).

Table 1. Description of the genotyping SNP assay for rs10757278 and rs2383206 in addition to the thermal profile for PCR ampilification

dbSNP ID	**rs10757278**	
Assay ID	C__11841860_10	
Location (NCBI Build 37)	Chr.9:22124477	
Species	Homo sapiens	
Set membership	HapMap	
Context sequence [(VIC/FAM)]	AAGTCAGGGTGTGGTCATTCCGGTA**[A/G]**GCAGCGATGCAGAATCAAGACAGAG	
Polymorphism	A,G transition substitution	
dbSNP ID	**rs2383206**	
Assay ID	C__1754669_10	
Location (NCBI Build 37)	Chr.9:22115026	
Species	Homo sapiens	
Set membership	HapMap	
Context sequence [(VIC/FAM)]	TTTTCCTTAGAAATGTTATTGTAGT**[A/G]**TTTGCAAGATGGCCTGAATCCTGAA	
Polymorphism	A,G transition substitution	
Thermal profile for PCR amplification		
Temperature (°C)	**Time (min:s)**	**Cycles**
95	10:00	Hold
92	00:15	
60	1:30	40

Table 2. The general characteristics of studied controls, cardiovascular patients, and cardiovascular diabetic patients

Groups	**Controls (n=50)**	**CAD patients (n=50)**	**CAD T2D patients (n=50)**	***P***
Age	32.38±1.23 (22–55 y)	43.62±2.01 (23–65 y)	50.12±1.29 (26–55)	0.37
Sex				
Male	38 (76%)	32 (64%)	34 (68%)	0.37
Female	12 (24%)	18 (36%)	16 (32%)	
SBP	121.4±0.948	**127.1±1.75****	**127.9±2.148**	**0.03**
DBP	80.3±0.720	82.4±1.118	81.34±1.418	0.15
Smoking, %	72%	26%	54%	0.37
BMI, kg/m^2	27.84±1.071	27.96±0.5291	29.28±0.6646	0.06
STC, mg/dL	151.3±15.43	180.7±17.8	**196.3±12.08**	**0.03**
Serum hs-CRP, mg/L	3.832±0.556	**7.207±0.419**	**8.895±0.355**	**0.00001**
HbA1c levels (%)	5.031±0.129	5.091±0.141	**7.274±0.292****##	**0.001**

Data are expressed as mean±SEM and compared using one-way ANOVA. Bold data show significant difference from controls at $P<0.05$. BM I- body mass index; CAD - coronary artery disease; DBP - diastolic blood pressure; Hb A1c - glycated hemoglobin; hs-CRP - high sensitivity C-reactive protein; SBP - systolic blood pressure; STC- serum total cholesterol; T2D - type II diabetes

Comparing the individual groups (CAD and CAD T2D) with controls yielded significant differences for CAD vs. controls genotypes GG (p=0.0014), AG (p=0.002), and AA (p=0.0004) with odds ratio (95% confidence intervals) for GG vs. AA, 4.52 (1.7–12.03) and for AG vs. AA, 5.60 (2.039–15.38) and for alleles (p=0.071) with odds ratio (95% confidence intervals) 1.729 (0.951–3.141).

For CAD T2D vs. controls, the p value for GG was 0.006, AG was 0.007, and AA was 0.001 with odds ratio (95% confidence intervals) for GG vs. AG, 3.261 (1.332–7.98) and for AG vs. AA, 4.2 (1.661–10.62), and for alleles it was 0.135 with odds ratio (95% confidence intervals) 1.565 (0.868–2.824).

Moreover, in harmony with rs10757278 results, no significant difference was found upon comparing the genotype or allele distributions of CAD patients with those of CAD T2D patients.

Association of genotypes with the biochemical data

No significant difference was found between the SNP genotypes and the biochemical markers in any of the studied groups for both SNPs (Tables 4).

Table 3. Genotypic and allelic distributions of all subjects in rs10757278 and rs2383206 SNP assays

	Controls (n=50)	CAD Patients (n=50)	CAD T2D patients (n=50)	*P*
rs10757278				
GG	13 (26%)	17 (34%)	20 (40%)	**0.0009*****
AG	18 (36%)	26 (52%)	19 (38%)	
AA	19 (38%)	7 (14%)	11 (22%)	
G	44 (44%)	60 (60%)	59 (59%)	**0.04***
A	56 (56%)	40 (40%)	41 (41%)	
rs2383206				
GG	23 (46%)	26 (52%)	25 (50%)	**0.001*****
AG	15 (30%)	21 (42%)	21 (42%)	
AA	12 (24%)	3 (6%)	4 (8%)	
G	61 (61%)	73 (73%)	71 (71%)	0.15
A	39 (39%)	27 (27%)	29 (29%)	

Association between the investigated polymorphisms and CAD was based on both genotypic and allelic models and was evaluated using the χ^2 test. *Significant difference between the three groups at $P<0.05$. ***Significant difference between the three groups at $P<0.001$. CAD - coronary artery disease; T2D - type II diabetes

Discussion

The present study evaluated the association of rs10757278 and rs2383206 SNPs on Chr9p21 with the incidence of CAD in the presence and absence of T2D. It also evaluated the relationship of the aforementioned SNPs with several biomarkers (TC, hs-CRP, and HbA1c). CAD, including its acute complication of MI, is the leading cause of mortality worldwide (18). It remains a serious clinical and economical problem, especially in Egypt where CVDs are among the main causes of hospitalization, disability, sick leaves, and premature mortality. CAD is a multifactorial disorder, and its phenotype results from the interactions between multiple genetic and environmental factors (19, 20). Till date, many SNPs of candidate genes and of regulatory loci have been discovered (21, 22), but the responsible molecular and genetic determinants remain largely veiled.

In the present study, significant differences in the genotype and allele distributions were observed between patients (CAD and CAD T2D patients) and controls in SNP1 (rs10757278). Similar observations were found in several studies of different populations (23–26). These results emphasize the association of G allele and GG genotype of SNP rs10757278 with the incidence of CAD. The frequency of A and G alleles in the Egyptian population is similar to that observed in the polish (23), German (24), Iceland, and U.S. populations (27). Another study also proved that the homozygous GG genotype of rs10757278 is associated with CAD severity, extent, and progression in Caucasian populations (25). Authors also noted a significant association in the prevalence of MI, with increasing copies of the risk allele (p=0.04) equating to an allelic OR of 1.18 (95% CI 1.04–1.34). Another study concluded that the risk allele, rs10757278(G), showed an increased association with MI and heart attacks both in general and, more specifically, in so-called early-onset MI (28). Furthermore, Tian et al. (29) showed that the variant GG genotype was associated with an increased risk of peripheral artery disease as well as CAD. Holdt et al. (30) showed that carrying 1 or 2 copies of chr9p21 risk allele confers more severe CVD, while Patel et al. (25) noticed faster progression of CVD in the risk allele carriers than in individuals with 0 copies. On the other hand, a study showed that chr9p21 locus was not associated with incident events or prevalent MI, although it did predict CAD diagnosis (31).

Regarding the second SNP, rs2383206, this study showed significant differences in genotypes distribution among CAD patients, CAD T2D patients, and controls. Similar results were observed in a study by Doria et al. (26). Our results markedly demonstrate that the genotype AA is protective from CVD. However, allelic variations were not statistically significant, most probably due to the sample size. Our study is in harmony with that of McPherson et al. (32) who found that the SNP rs2383206 allele was associated with an approximately 15%–20% increase in the risk of CHD in heterozygous Caucasians patients and a 30%–40% increase in the homozygous patients. In the study by Doria et al. (26), individuals who were homozygous for the risk allele were significantly more frequent among patients than among control participants (p=0.0002), but the effect of the risk genotype was significantly magnified in the presence of poor glycemic control. This suggested a potential interaction between the chr9p21 CAD haplotype and diabetes mellitus in the development of CAD in Chinese diabetic patients, compared with non-diabetic CAD patients (33).

Some studies showed a strong linkage disequilibrium (LD) between the two SNPS, rs10757278 and rs2383206 (26, 34). Doria et al. (26) showed that the G allele of rs2383206 was associated with CAD regardless of the rs10757278 allele that was present on the same haplotype, whereas the A allele of rs10757278 was protective only when it occurred together with the protective allele of rs2383206. Our study showed LD for both SNPs (D'=0.8, r2=0.4). This supports the outcomes of our study that showed similar results for both SNPs regarding association with CVD and insignificant effect on the biochemical data.

The association of the two SNPs rs10757278 and rs2383206 on the susceptibility locus 9p21 with CAD in multiple cohorts of European descent was demonstrated by Shen et al. (35). Beckie et al. (34) examined the allelic frequencies and haplotype structure of genetic variants on chromosome 9p21 in a cohort of black and white women with CHD and examined the relationship of these genetic variants with the age of onset of CHD. Few blacks as a different from whites, had the risk genotype for rs10757278 SNP, and only 12.5% of blacks had the risk genotype (GG) for rs2383206 compared with 35.2% of whites (34). Another study by Beckie et al. (36), comparing white women to black ones, found few blacks with the homozygous risk alleles of rs10757278-G and rs2383206-G.

The serum total cholesterol levels showed significant difference between the three groups (controls, CAD patients, and CAD T2D patients). However, all levels lay within the normal range

Table 4. Association of rs107572728 and rs2383206 genotype with biochemical markers in the studied groups. Data are expressed as mean±SEM

	Biomarker		GG	AG	AA	*P*
rs10757278	Serum total cholesterol, mg/dL	Control	141.7±13.71	157.1±32.40	152.4±26.25	0.89
		CAD	269.7±36.03	195.7±21.88	141.4±33.41	0.073
		CAD T2D	204.2±19.23	169.5±19.43	199.7±33.06	0.41
	Serum hs-CRP, mg/L	Control	4.213±1.06	2.601±0.86	5.09±0.89	0.05
		CAD	7.65±0.70	6.99±0.55	7.11±1.62	0.75
		CAD T2D	9.644±0.181	8.435±0.707	8.112±1.100	0.21
	HbA1c (%)	Control	5.09±0.31	5.02±0.20	5.01±0.24	0.94
		CAD	5.26±0.29	5.02±0.19	4.99±0.13	0.66
		CAD T2D	7.33±0.37	6.73±1.02	7.59±0.35	0.75
rs2383206	Serum total cholesterol, mg/dL	Control	150.4±13.85	168.4±37.05	130.2±36.10	0.39
		CAD	215.4±27.27	190.5±23.64	101.3±9.21	0.25
		CAD T2D	206.3±17.99	172.5±21.27	202.7±13.28	0.39
	Serum hs-CRP, mg/L	Control	4.00±0.86	4.54±1.11	3.18±1.01	0.54
		CAD	7.38±0.53	7.17±0.66	5.94±3.46	0.77
		CAD T2D	9.67±0.17	8.30±0.64	7.86±2.00	0.08
	Glycated hemoglobin HbA1c (%)	Control	5.02±0.23	5.10±0.14	4.97±0.28	0.86
		CAD	5.31±0.22	4.81±0.16	5.12±0	0.16
		CAD T2D	7.38±0.37	7.14±0.62	7.22±0.65	0.98

The association of the three different study groups with the three biomarkers (TC, hs-CRP, and HbA1c) was tested using nonparametric one-way ANOVA (Kruskal–Wallis test), followed by Dunn's multiple post-test to compare between each two groups. CAD - coronary artery disease; HbA1c - glycated hemoglobin; hs-CRP - high-sensitivity C-reactive protein; T2D - type II diabetes

(140–200 mg/dL); this can be explained by the fact that the majority of recruited patients were in-patients, and they were taking hypolipidemic drugs. Regarding the serum hs-CRP levels in this study, significant differences were observed between the three groups, with p=0.0001. The significant difference between CAD T2D patients and CAD patients indicates that diabetes aggravates the inflammation associated with CAD (37–39). Current evidence suggests that inflammatory and metabolic factors associated with diabetes such as high glucose, adipokines, modified lipoproteins, and free fatty acids may trigger hs-CRP production by endothelial cells, smooth muscle cells, and monocytes/macrophages (37). The glycated hemoglobin levels in our study showed significant differences between the three groups, and CAD T2D patients showed the highest levels that were still under fair control.

Study limitations

Sample size limitations and controls were not subjected to coronary angiography for confirmation of the exclusion of coronary lesions.

Conclusion

We can conclude from this study that rs10757278/rs2383206-G allele increases the risk for CAD in Egyptians. Moreover, AA variant appeared as a protective genotype. However, SNPs did not noticeably contribute in the elevation of TC, hs-CRP, and HbA1c levels in non-diabetic and diabetic CAD patients. These biochemical parameters were significantly elevated in CAD T2D patients compared with CAD patients and controls.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – M.Z.G., S.I.H.; Design – M.Z.G., S.I.H., H.A.S.; Supervision – M.Z.G., S.I.H.; Fundings – M.Z.G., S.I.H.; Materials – H.A.S.; Data collection and/or processing – H.A.S., S.I.H.; Analysis and/or interpretation – M.Z.G., S.I.H., H.A.S.; Literature review – H.A.S.; Writing – M.Z.G., S.I.H.; Critical review – M.Z.G.

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