Investigate the relation between Adiponectin gene variants and cardiovascular comorbidities and diabetes

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Abstract

Objectives: This study aimed to study the relationship between the adiponectin gene and coronary artery disease as well as myocardial infarction, hypertension and diabetes in the Saudi population in Riyadh.

Methods: This was a cohort study of Saudi patients from the Coronary Artery Disease (CAD) registry maintained at King Faisal Specialist Hospital and Research Centre. Samples were genotyped for target Single Nucleotide Polymorphisms (SNPs) by real time polymerase chain reaction (PCR). Furthermore, Chi Square test was used for comparing the variables.

Results: Out of 860 CAD patients compared with 467 non-CAD. The prevalence of the minor G allele of +45T>G in CAD patients was 934 compared to 786 in controls, and it shows no significant difference (p=0.598) between the two groups. The possibility tested that this variant might be associated with one or more of the CAD risk factors, such as hypertension, myocardial infarction, hypertension and diabetes mellitus. Nevertheless, the study did not show any significance between the two groups in CAD, gender and hypertension. On the other hand, the difference in MI was borderline and significant in diabetes mellitus.

Conclusions: This study showed a relationship between adiponectin and diabetes only as compared to results from other countries which also showed relationship with coronary artery disease, myocardial infarction and hypertension.

Key words: Adiponectin, Cardiovascular, genetics, Diabetes

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Introduction

Adiponectin or *ADIPOQ* gene is the most abundant adipocytokine (0.01% of plasma protein) that is secreted by adipocytes. [1,2] Over the past few years, It has drawn the awareness for its potential role in the diabetes mellitus, [3] metabolic syndrome (4), and coronary artery disease (CAD) among different populations. [5, 6] It is encoded by the *ADIPOQ* gene (ENTREZ Gene ID: 9370), [7] which consists of 3 exons and 2 introns spanning a 17-kb region that is located on chromosome 3q27. It is a 244 amino acid protein solely which is derived from the adipose tissue. [8, 9] This chromosomal region was identified in genetic linkage studies to identify a diabetes susceptibility locus (10). A recent comprehensive review shows that few *ADIPOQ* Single Nucleotide Polymorphisms (SNPs) are associated with plasma adiponectin and insulin levels, however it is not consistent. [11] Two receptors are thought to be mediating the adiponectin action in fatty acid oxidation and glucose uptake, namely *ADIPOR1* and *ADIPOR2.* [12]

Some articles suggested that adiponectin has anti-inflammatory, insulin sensitizing and atherogenic effects on mice. [1, 2] An administration of recombinant adiponectin promotes lipid beta oxidation in skeletal muscles, [13] reduces hepatic gluconeogenesis [14] and improves glucose tolerance and insulin sensitivity in humans. [15] Gene variations lead to changes in the gene sequence, such as the variations in the exon (coding) can cause structural and possibly functional changes in the gene. Many variations in the adiponectin gene were linked with different disorders, such as Type 2 Diabetes Mellitus T2DM, [3] metabolic syndrome [4] and CAD. [5, 6] The *ADIPOQ* rs2241766 (+45T>G in exon 2) was significantly associated with the risk of T2DM in Japanese, [16] however the association with the French populations was not significant. [17] In the Germany population, the SNP with the G/G genotype at position +45T>G was dramatically associated with the obesity and insulin resistance. [18] These results represent the variety of adiponectin SNP45 among different population (Table1) and it how may play a role in the association between some diseases and certain ethnic groups. The *ADIPOQ* SNP 276G>T was found to be related to insulin resistance and CVD risk. [19] Another variant, Y111H was found to be a causative factor for T2DM. [20] Furthermore, the SNPs in the promoter region (-11426A/G,-11377C/G and -11391G/A) was related to T2DM. [20] A clinical study on Han Chinese women found a significant correlation between polycystic ovarian syndrome and the SNPs +34G15G (T/G) and +276(G/T) with. [21]

In the Saudi population, SNPs 45T>G and 276G>T of *ADIPOQ* gene were assessed. Diabetic patients with 45T>G polymorphism had significant association with the incidence of CAD. However, 276G>T of the diabetic patients was not associated with CAD significant statistically. [22] On the other hand, in another study, none of the SNPs 45T>G and 276G>T are not significantly related to obesity, Type 2 Diabetes Mellitus, lipid profile and Hypertension in a Saudi population. The aim of the study is to identify the relationship between the adiponectin gene and coronary artery disease, myocardial infarction, hypertension and diabetes in the Saudi population. [23]

Subjects and Methods

Study population:

A cohort study of 860 Saudi individuals from the CAD registry maintained at King Faisal Specialist Hospital and Research Centre comprised the study group. Among these, 467 had clinical manifestations of CAD, while 393 had no significant coronary stenosis by angiography.

Five ml blood was sampled from study individuals after obtaining their written consent, and DNA isolated using the puregene DNA isolation kit (Qiagen Sciences, Maryland, USA). Briefly, erythrocytes were lysed in RBC lysis buffer, and the proteins removed by precipitation in TCA buffer. The DNA was subsequently isolated by precipitation in alcohol, quantified and stored at -80°C if not required immediately.

Samples were genotyped for target SNPs by real time PCR using the 7900HT Sequence Detection System (ABI, Foster City, CA, USA). Primers and TaqMan probes were designed using the Primer Express software V2.0 (ABI, Foster City, CA, USA) and obtained from Applied Biosystems (Warrington, UK). The fluorogenic probes, bearing a suitable reporter dye on the 5' – end and a quencher dye on the 3' –end, hybridize to the specific sequence to be amplified by the PCR primers. The quencher

prevents fluorescent emission from the reporter dye when the probe is intact. When the probe is cleaved by the polymerase exonuclease activity in the extension phase of PCR, there is increased fluorescent emission from the reporter which is no longer in close proximity to the quencher.

One probe, for allele 1, was labelled with VIC dye and the other, for allele 2, with FAM dye at the 5' –end. Serial dilutions of the probes were run to determine the optimal working concentration. For each well of a 96-well reaction plate a 25 µl reaction was prepared by mixing 5 µl containing 50ng DNA, 12.5 µl of 2x universal mix (Eurogentec, liege Science Park, 4102 Seraing, Belgium), and 1.25 µl of 20 x probe Assay mix and 6.25 µl of 1 x Tris EDTA buffer. Three no-template controls were included for each plate.

The thermal profile for amplification was a 1st cycle at 50°C for 2 minutes, and 95°C for 10 min, followed by 40 cycles of 94°C for 15sec, and 60°C for 30 sec (Qiagen GmbH, Hilden, Germany).

The plate wells were scanned for FRET signal in the 7900HT sequence detection system, and the data analyzed using SDS 2.0 software (ABI, Foster City, CA, USA).

Data analysis was done by using SPSS 22.0 statistical software for Windows. Quantitative variables are presented as mean and standard deviation. Qualitative variables are presented as frequency and percentages. Chi Square test was used for comparing the categorical variables. For the comparison, two-tailed *p* value <0.05 was considered statistically significant.

Results

In the study, the total population was 860 from CAD registry, this comprised of 580 (67%) males and 280 (33%) females. The mean age of the subjects was 553 $\pm$ 14 years with an age range of 16-95 years old. Two alleles were examined for each subject making a total of 1720 alleles. They were 1371 (80%) VIC dye and 349 (20%) FAM dye (Table 2). Out of the total subjects, 393 (46%) had CAD, 484 (58%) had diabetes, 631 (76%) had hypertension and 664 (77%) had myocardial infarction. There was no difference in the distribution of alleles by gender with 930 (80%) of the 1160 male alleles have been VIC as compared to 441 (79%) of 560 females alleles (p=0.49).

Table 3 shows the distribution of VIC and FAM alleles in relation to different diseases. There was no association of VIC or FAM with CAD (p=0.58) or hypertension (p=0.14). In subjects with Diabetes Mellitus (DM), out of 964 alleles, 219 (23%) were FAM as compared to 124 (18%) out of 706 alleles of non-diabetic subjects (p=0.01). there was also a borderline association between Myocardial Infarction (MI) and FAM with 282 (21%) out of 1328 alleles of MI patients being having FAM as compared to 67 (17%) of 383 alleles in subjects without MI (p=0.09).

Table1: polymorphism of certain SNPs of the adiponectin

SNP Locus	SNP ID	Relation to Gene	Minor Allele	MAF (HapMap)	
				CEU	HCB
-11426A/G	Rs16861194	Promoter	G	0.067	0.167
-11391G/A	Rs17300539	Promoter	A	0.083	0.000
-11377C/G	Rs266729	Promoter	G	0.308	0.300
+45T/G	Rs2241766	Exon 2 coding synonymous	G	*0.056	--
+276G/T	Rs1501299	Intron 2	T	--	--
Y111H	Rs17366743	Intron 2	T	0.925	1.000

*according to pilot 1

Table 2: Demographic data

Age (n=859)	Mean	sd
Mean	55.3	± 14.0
	n	%
Sex (n=860)		
Male	580	67.4
Female	280	32.6
CAD (n=860)		
CONTROL	393	45.7
CAD patients	467	54.3
DM (n=835)		
CONTROL	353	42.3
DM patients	482	57.7
HTN (n=833)		
CONTROL	202	24.2
HTN patients	631	75.8
MI (n=858)		
CONTROL	194	22.6
MI patients	664	77.4

CAD: Coronary Artery Disease
DM: Diabetes Mellitus
HTN: Hypertension
MI: Myocardial Infarction

Table 3: The analysis compares 467 coronary artery disease cases versus 393 controls. *P-value by X^2 test.

	Total	VIC	FAM	p-value
CAD (n=860) x2				
Patients	934	740 (79%)	194 (21%)	0.58
Controls	786	631 (80%)	155 (20%)	
HTN (n=833) x2				
Patients	1262	1015 (80%)	247 (20%)	0.14
Controls	404	311 (77%)	93 (23%)	
MI (n=858) x2				

Patients	1328	1046 (79%)	282 (21%)	0.09
Controls	388	321 (83%)	67 (17%)	
DM (n=826) x2				
Patients	946	745 (79%)	219 (23%)	0.01
Controls	706	582 (82%)	124 (18%)	

CAD: Coronary Artery Disease
DM: Diabetes Mellitus
HTN: Hypertension
MI: Myocardial Infarction

Discussion

Adiponectin was found to play a momentous role in the diabetes mellitus, metabolic syndrome, and coronary artery disease among different populations according to other studies. [5, 6] Furthermore, SNPs 45T>G polymorphism of the diabetic Saudi population had a higher tendency of having coronary artery disease. [22] However, the same SNPs are not significantly linked to obesity, T2DM, hypertension and lipid profile according according to Al-Daghri's results. [23] On the other hand, the results of the present study suggested that there is no significant relationship between the +45T>G of the *ADIPOQ* and cardiovascular conditions in Saudi population. Yet it indicated a relation between reduction of *ADIPOQ* level in plasma or serum and diabetic patients (p=0.01). These findings are inconsistent with several studies which suggested that this SNP polymorphism is a risk for various cardiovascular disorders. Additionally, the results are incompatible with two studies on Saudi populations. Firstly, (Al-Daghri et al 2011) found that there is a significant association between SNPs 45T>G polymorphism in diabetic Saudi population with CAD. [22] Secondly, (Al-Daghri et al 2012) stated that there is no relation between this SNPs and obesity, T2DM, hypertension and lipid profile. [23]

The prevalence of diabetes in Saudi population is high and it is considered as one of the major public health problem. One of the studies published that 30% of 6024 Saudi subjects diagnosed with Diabetes. [24] Moreover, type 2 Diabetes Mellitus is found to be a multifactorial condition that is caused by genetic and environmental factors. [25] For that reason, Adiponectin may play a major role in Diabetes mellitus, however other factors should be taken in consideration.

This study can attract clinicians and researchers to investigate more in this SNP and many others to find some correlations with diseases that are idiopathic and do not have clear aetiologies. However, the possible limitation of this manuscript is that the sampling depended on the availability of the samples and the time interval for this project.

Conclusion

The present study indicates an association between adiponectin and diabetes, as well as borderline association with myocardial infarction. Nevertheless, it does not show a relationship with coronary artery disease and hypertension. The findings are conflicting with other studies.

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