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Association of a *Disintegrin and Metalloproteinase 33* Gene Polymorphisms with Chronic Obstructive Pulmonary Disease in Iraqi Population

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Abstract

Background:
"Chronic obstructive pulmonary disease (COPD) is predisposed by environmental and hereditary factors *A disintegrin and metalloproteinase 33* gene (*ADAM33*) has been one of the most stimulating gene for asthma since of their first association with the disease in Caucasian population . Recently, *ADAM33* was shown to be associated with decrease of lung function and COPD. The target of this study was to evaluate the potential correlation between polymorphisms of ADAM*33* and COPD in Iraqi population.

Methods:
This study included, 400 cases of COPD and 400 healthy individuals as control group. "Two polymorphic loci (V4 and Q-1) of ADAM*33* were selected for genotyping that determined by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method

Results:
"Statistically significant distinctions in the distribution of the wild and mutant genotypes between patients and control. In addition, significant association (P <0. 0001) between COPD and mutant genotypes (V4/GG and Q-1/AA) are detected.

Conclusion:
The results of this study designate that *ADAM33*/V1 and Q-1 polymorphisms is a hazard factor for COPD among Iraqi society.

Key words: COPD, *ADAM33*, Polymorphism, Genotype

INTRODUCTION

Chronic obstructive pulmonary disease is characterized by the development of airflow limitation that is progressive and not completely reversible [1, 19], and is a major and growing public health burden as the 4th leading cause of loss of life in the world regarding to 2002 statistics [2]. Chronic obstructive pulmonary disease is estimated to be one of the main five chronic diseases in terms of global mortality and morbidity by 2030 [3]. The relationship between gene polymorphisms and COPD susceptibility has recently been paid special attention and was explored in a large number of studies on hundreds of genes, however the results varied between studies and populations of people [8]. Multiple genes have been found for asthma and COPD. In addition to genes unique to these diseases, some shared genetic risk factors exist. Moreover, there are common host risk factors and environmental risk factors for asthma and COPD. Based on the data available, some studies put forward that genes that affect lung development in utero and lung growth in early childhood in interaction with environmental detrimental stimuli, such as smoking and air pollution, are contributing to asthma in childhood and the ultimate development of COPD [16]. *A disintegrin and metalloproteinase 33* gene, belonging to the disintegrin and metalloprotease family, plays a vital role in cell adhesion, proliferation, difference, signaling, apoptosis, and other responses [4, 20]. van Eerdewegh *et al.*, (2002) found a relationship between the ADAM*33* gene with asthma and bronchial hyper responsiveness. In the past decade, an increasing number of studies have shown associations between *ADAM33* polymorphisms and asthma susceptibility, as well as other pulmonary diseases in several populations [5,6,7,15]. van Diemen *et al.*, (2005) reported, for the first time, that single nucleotide polymorphisms (SNPs) in *ADAM33* were associated with accelerated lung. The present study is the first attempt to detect the association between *ADAM33*/V4 and Q-1 polymorphisms and COPD in Iraqi population.

MATERIAL AND METHODS

Study design: The current study was conducted on 400 patients (237males, 163 females) their ages from 30-80 year were seen in Al-Diwaniya Teaching Hospital. The patients were diagnosed clinically by physician as having COPD. The diagnosis of COPD performed by using special criteria or standards: {1} record of cigarette smoking (at least for 9 years ago) in patients who were current smokers at the time of evaluation {2} not exposition to other substances that identified to cause lung abnormalities {3} nonexistence of atopy {4} no history of systemic or other pulmonary disease or congenital and /or acquired systemic immunodeficiency {5}forced expiratory volume in the first second (FEV1)/forced vital capacity (FVC) <70% and FEV1 after inhalation of 200 mg salbutamol <80% [9]. Another group consist of 400 apparently healthy individuals (210 males and 190 female) their ages from 30-80 year without smoking or any history of systemic disease were clinically considered as a control group.

Molecular study: genomic DNA was extracted from blood according to manufacturers' instructions of Genomic

DNA Mini Kit (Geneaid). The ADAM*33* was amplified by PCR by using Professional TR/O Thermocycler and information on the chosen SNPs is exposed in table 1. In RFLP technique, the PCR product were digested overnight with restriction enzymes (Table 1) according to manufacturers' procedure, and analyzed by 2% agarose gel electrophoresis

Statistical analysis: Statistical analyses were performed by the Statistical Package for Social Sciences version 20 for Windows software and Microsoft Excel 2010. Strong correlation between variables are determine by OR and $\chi 2$ test or Fisher's exact test. A *P* -value of <0.05 was considered to be statistically significant

RESULTS

The presence of family history is an important contributory factor in COPD. This study showed 260 (65%) of COPD patients have positive family history, Figure (1). The distribution of ADAM*33* polymorphism was detected by PCR -RFLP technique. *ADAM33*-V4C/G locus have three genotypes; GG, GC and CC with band sizes of 168/206 bp, 168/206/374 bp and 374 bp respectively (Figure 2). Also in ADAM*33*-Q-1 A/G locus there're three genotype; GG, AA and AG with band sizes of 158 bp, 138/20bp and 158/138/20 bp respectively as in Figure (3). Deviations from Hardy-Weinberg equilibrium for *ADAM33*/Q-1 and V4 polymorphisms were seen in the COPD or healthy group. The genotypes and alleles frequencies of the ADAM33 SNPs in the COPD and healthy group are revealed in table 2. Mutant homozygous genotype (GG) of *ADAM33*-V4 and Mutant homozygous genotype of *ADAM33*/Q-1(AA) significantly association with COPD in compared with control (p <0.0001). *ADAM33*/Q-1(AA) and ADAM*33*-V4(GG) genotypes appeared as risk factors in COPD (EF = 0.329 and EF =0.446 respectively). In contrast, wild homozygous genotype (CC) of *ADAM33*-V4 and wild homozygous genotype of *ADAM33*/Q-1(GG) mainly detected in control group (p <0.0001) and not have any risk in COPD but wild homozygous genotypes of *ADAM33* SNPs have Protective role (PF=0.328 and PF= 0.326 for V4/CC and Q-1/GG respectively). Heterozygous genotype of *ADAM33* SNPs (V4/CG and Q-1/AG) not considerably association with COPD (p>0.05) but Q-1/AG act as moderate risk factors (EF =0.088). *ADAM33*/Q-1(A) and *ADAM33*-V4(G) alleles are Mutant alleles and significantly association with COPD in compared with control (p< 0.0001) also appeared as risk factors (EF=0.487 and EF=.0.569 for V4/G and Q-1/A respectively).

Table (1): SNP characterized, Sequences of primers and Restriction Enzymes for *ADAM33* genotyping.

chromosome position	Reference SNP ID	SNP name	Allele	Primer sequence	Restriction enzyme
3592207	612709	Q-1	A/G	F: 5'-GGATTCAAACGGCAAGGAG-3' R: 5'-GTTCACCTAGATGGCCAGGA-3'	PstI [9]
3589161	2787094	V4	C/G	F: 5'-ACACACAGAATGGGGGAGAG-3' R: 5'-CCAGAAGCAAAGGTCACACA-3'	BtsCI [9]

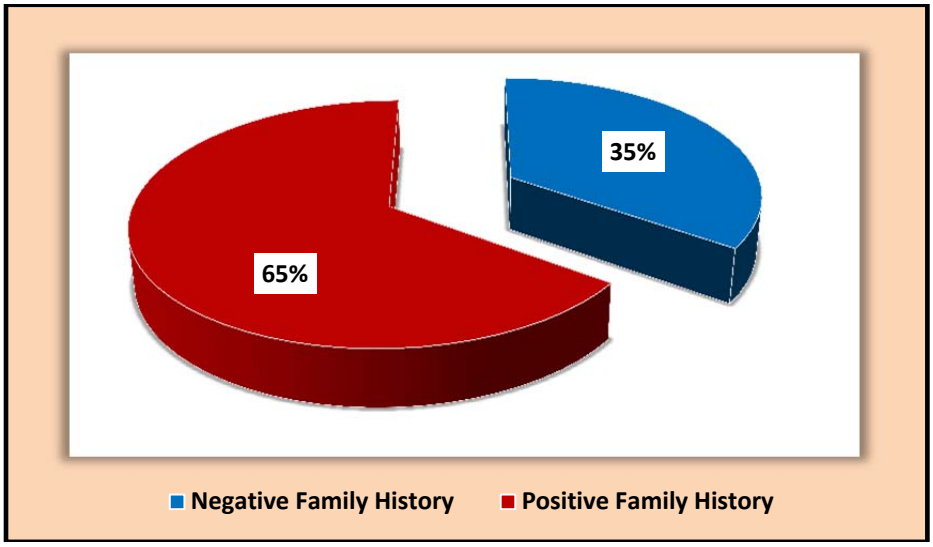

Figure (1): A Pie Chart showing the association between family history and COPD in **cases.**

Table (2): prevalence of genotypes and alleles of ADAM*33/V4* and *Q-1* in case-control

SNPs	Genotype/ Allele	Healthy controls (n=400)		Cases (COPD) (n=400)		Case-control comparison					
	Genotype	N	%	N	%	P value	OR	95% CI OR	X^2	EF	PF
ADAM33-V4 (rs2787094)	GG	41	10.2	201	50.3	<0.0001	8.844	(6.06 - 12.91)	151.7	0.446	**
	CG	162	40.5	160	40	0.888 [NS]	0.969	(0.731 - 1.286)	0.021	**	0.013
	CC	197	49.3	39	9.7	<0.0001	0.166	(0.114 - 0.242)	150.04	**	0.328
	Allele										
	G	244	30.5	562	70	<0.0001	5.381	(4.346 - 6.66)	252.8	0.569	**
	C	556	69.5	238	30	<0.0001	0.186	(0.15- 0.23)	252.8	**	0.568
ADAM33-Q1 (rs612709)	Genotype										
	AA	99	24.8	198	49.5	<0.0001	2.98	2.208-4.022	52.49	0.329	**
	AG	150	37.5	172	43	0.068 [NS]	1.257	0.947-1.669	3.338	0.088	**
	GG	151	37.7	30	7.5	<0.0001	0.134	0.088-0.204	104.54	**	0.326
	Allele										
	A	348	43.5	568	71	<0.0001	3.179	2.585-3.911	123.6	0.487	**
	G	452	56.5	232	29	<0.0001	0.315	0.256-0.387	123.6	**	0.387

❖ **OR=Odd ratio, EF= Etiology fraction, PF=Preventive fraction, NS= No significant (p >0.05).**

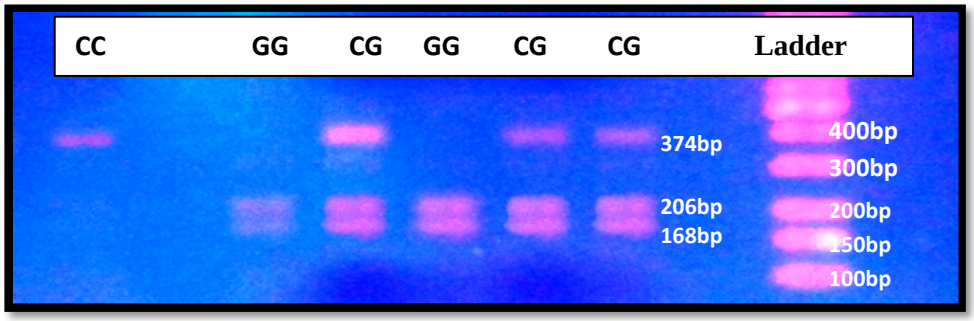

Figure (2): Agarose gel electrophoresis image that show the PCR - RFLP product of ADAM*33/V4* polymorphism by using *PstI* restriction enzyme.

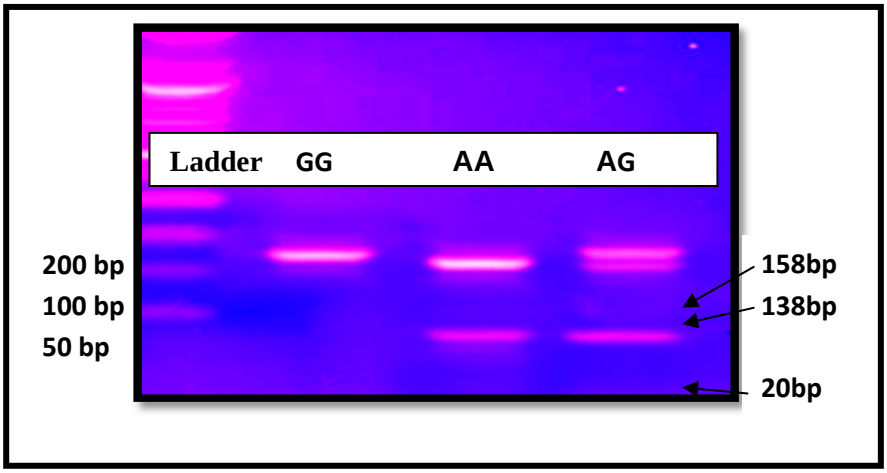

Figure (3): Agarose gel electrophoresis image that show the PCR - RFLP product of ADAM*33/Q-1* polymorphism by using *BtsCI* restriction enzyme.

DISCUSSION

Decline in lung function is a threat factor for the development of COPD and cardiovascular disease [10]. Lee and coworkers demonstrated that *ADAM33* protein levels in bronchoalveolar lavage fluid are inversely correlated with predicted FEV1 values in patients with asthma [11, 14]. Associations of polymorphisms in *ADAM33* with FEV1 decline may therefore represent a risk for the development of COPD as well [12] . In present study, *ADAM33* SNPs (Q-1 and V4) extensively association with

FEV1 decline and COPD and this agreed with Wang *et al*., (2009) who detected that the intrinsic Q-1 SNP may influence the splicing of the ss-variant and disturb the growth of *ADAM33*. Subsequent results on protease activity may cause a defect in tissue repair after inflammation-induced damage. This may lead to progressive damage of alveolar tissue and thereby enhance accelerated decrease in lung function [9]. In a Dutch population, the SNPs S1, S2, and Q-1 were associated with FEV1 decline, and the SNPs S1, S2, F+1, and T2 were associated with the presence of COPD while paper of Simpson *et al*., (2005) on European children showed that SNPs F+1, M+1, T1, and T2 significantly associated with lower FEV1 and COPD [13]. Current research in line with Xiao *et al*., (2005) who point out there is a relationship between *ADAM33*-V4 polymorphism and COPD in Tibetan population of china [17] but inconsistent with study of Pabst, *et al*., (2009) who show no genetic association between polymorphic variants in *ADAM33* and the onset or course of COPD [18,21]. Finally, only present study in Iraq are detected that *ADAM33*-V4 and Q-1 SNPs have role in development of COPD and other researches on genetics of COPD should be completed to determine the causative factors of COPD in this population.

CONCLUSION

COPD not causes by smoking or other environmental factors only but also strongly associated with genetic polymorphism. *ADAM33*/V4 and Q-1 SNP polymorphisms significantly correlated with COPD patients and this mean that individual who has *ADAM33*/V4 and Q-1 SNP polymorphisms more susceptible to COPD in this population.

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