Evaluation of A Disintegrin and Metalloprotein33 Gene Polymorphism in Bronchial Asthma

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الخلاصة:

الربو هو مرض اللتهابي مزمن، ينتج بصورة رئيسية عن التفاعلْ بين العواملِ الوراثيةِ والبيئيةِ. أجرتْ هذه الدراسةِ على (69) مريض (48 أنثى و 21 ذكر) مصاب بالربو القصبي، تتراوح اعمارهم من (18-70) سَنَة، مع (20) شخص معافى (11 أنثى و9 ذكورٍ) كمجموعة تحكم، راى في مستشفى الديوانية التعليمي من كانون الأولِ 2012 إلى كانون الثَّاني 2013. عينات الدم جمعت من كلتا المجموعتين، الحمض النووي استخرج من الكريات البيض للكشف عن وجود أي ارتباطات بين تعدد الإشكال الجينية ل *ADAM33* في الموقع 4 واستعداد الإصابة بالربو القصبي بواسطة تفاعلات البلمرة المتسلسله وتقنية الأجزاء المتكسرة المتعدد باستخدام إنزيم PstI والتي تعطي أجزاء ذات حجوم جزيئيه مختلفة تعبر عن تراكيب جينية معينة. بينتْ نَتائِجَ الدراسة بأنّ 31.9 % من المرضى في فئة عمر من (20-35) سَنَة و 27.5 % من المرضى كَانَ في فئة عُمرَ (20>) سَنَة، كذلك الدراسة بينت 69.6 % من المرضى كانوا إناث. كشفتْ دراسة ايضا ان نسبةَ إنتشارَ النكليوتيدِات الوحيدِة المتعدد الاشكال *ADAM33-V4* كَانتْ عاليةً جداً بين حالاتِ الربو ($P < 0.001$) ووَجدتْ ان ترددات التراكيب الوراثية متخالف اللواقح GG و متحول متماثل اللواقح CG والاليل المخالف G كَانتْ عالية جداً في المرضى المصابون بالرَّبوِ ($P <0.001$, $P = 0.023$, $P <0.001$ على التوالي) على النقيض من ذلك التركيب الوراثي متماثل اللواقح الطبيعي CC ($P= 0.451$) والاليل C ($P=0.6$) لَيْسَ لَها علاقة واضحة مَع المرضى المصابون بالرَّبوِ. اظهرت البياناتَ بأنّ 65.2 % مِنْ المرضِى المصابون بالرَّبوِ يمتلكُون تأريخُ عائلي إيجابيُ وبينت بأنّ التركيب الوراثي متخالف اللواقح GG والاليل G إرتبطَ بشكل ملحوظ بالمرضى الذين يمتلكون تأريخ عائلي للربو (56.70 % و75 % على التوالي) كما هو مُقارن مَع المرضى بدون تأريخ عائلي للربو (21.70 % و47.8 %)، بينما التركيب الوراثي متماثل اللواقح الطبيعي CC و التركيب الوراثي متحول متماثل اللواقح CG و الاليل C ليس مرتبط مَع المرضى المصابون بالرَّبوِ الذين امتلكوا تأريخ عائلي (8.7%، 32.6 % و25% على التوالي).

Abstract:

Background: A disintegrin and metalloprotein 33 (ADAM33) gene is the first asthma candidate gene identified by positional cloning, may be associated with lung function decline and bronchial hyperresponsiveness. However, replication results have been inconclusive in smaller previous study populations probably due to inconsistence in asthma phenotypes or yet unknown environmental influences. This study aimed to further elucidate the role of ADAM33 polymorphisms (SNPs) in a genetic analysis of our case- control. Materials and methods: One polymorphic sites (V4) of ADAM33 gene was genotyped in 69 patients with bronchial asthma, and 20 healthy controls. Genotypes were determined by the polymerase chain restriction fragment length polymorphism (PCR-RFLP) method. Data were analyzed using the chi-square test and SPSS version 20 computer software (Statistical Package for Social Sciences) in association with Microsoft Excel 2010. Results: The single nucleotide polymorphisms V4 G/C, of the ADAM33 gene may be participate in the susceptibility of bronchial asthma in the Iraqi population. Conclusion: Although ADAM33-V4 polymorphism not associated with asthma in many population, our study confirmed significant correlation between ADAM33-V4 and asthma .

Keywords: Asthma; Genetic; A disintegrin and metalloprotein 33; Polymorphism; Genotype; Allele.

Introduction:

Asthma is a chronic inflammatory disease of the airways of unknown etiology that is manifested as reversible airway obstruction **(1,2, 3)**. It is one of the most common chronic conditions, affecting at least 7-10 % of the adult population. Although most cases begin before the age of 25 years, asthma may develop at any time throughout life. Allergies are common in childhood-onset asthma but are somewhat less so in adult-onset disease **(4, 5,6)**. asthma has about 60% heritability, but it appears to be a complicated process with multiple involved genes and likely gene-environment interactions indicating that both genetic and environmental factors are important in its etiology **(7,8).** The most important environmental factors appear to be airborne allergens and viral infections. Diet, tobacco smoke, and air pollutants may also contribute to the development of asthma in susceptible persons **(9)**. Despite this evidence for a substantial genetic contribution to the biology of asthma and identification of a number of candidate genes, no discovered genetic variant has enhanced risk for the asthma phenotype across all populations. Studies suggest that multiple genetic variants account for the heritability of asthma in a given individual and that variations in different genes contribute to expression of the phenotype across a population. Genetic variants that influence the response to treatment also have been identified **(10,11).**

A disintegrin and metalloprotease domain 33 (*ADAM33*) protein associated with development of asthma **(12)**. Several single-nucleotide polymorphisms (SNPs) in the *ADAM33* gene promoter have been identified, some of which may regulate *ADAM33* expression and enhance its production in excess **(12, 13)**. *ADAM33* gene was the first putative asthma susceptibility gene to be identified by positional cloning in two Caucasian populations; one in the United Kingdom (UK) and the other in the United States of America (USA) **(10**). Since the first study on the association between *ADAM33* polymorphisms and asthma, several replication studies (including this study) have been published with conflicting results **(13)**. The goal of the study to Evaluate the role of *ADAM33*-V4 polymorphism in adult asthmatic patients and compared them with non-asthmatic persons by evaluation of the genotypic and allelic frequency.

Materials and methods

Subjects. The current study was conducted on 69 patients (48 males, 21 females) were seen in Al-Diwaniya Teaching Hospital from December 2012 to January 2013. The patients were diagnosed clinically by physician as having bronchial asthma. Patients were interviewed directly by using an anonymous questionnaire form which covered age, sex, duration of the disease, detail history about occupation, smoking, the frequency of symptoms, any drug use, family history and others. Another group consist of 20 apparently healthy individuals (11 male and 9 female) without any history of systemic disease were clinically considered as healthy also included in this study as a control group. This study was in agreement with ethics of Al-Diwaniya Teaching Hospital and verbal informed consent was obtained from all participants.

DNA extraction and genotyping. Genomic DNA was extracted according to the manufacturer's protocol from 5 ml of frozen whole blood using a DNA Extraction Kit (Geneaid / USA). Only V4 (rs2787094) SNP in the *ADAM33* gene was selected, which previously had been shown to have an allelic and/or

genotype association with Asthma. The polymorphic region was amplified by PCR. Amplification reaction were preformed in 0.2 ml tube of Accu Power PCR Premix tube according to the bioneer's corporation then the thermocycling condition for this reaction carried out and products analyzed by 1% agarose gel electrophoresis. PCR products were digested overnight with restriction enzymes (PstI), according to the manufacturer's protocol, and analyzed by 2% agarose gel electrophoresis.

Statistical analysis. Statistical analyses were done using SPSS version 20 computer software (Statistical Package for Social Sciences) in association with Microsoft Excel 2010. Odd ratio was used to measure the strength of association between 2 categorical variables and the statistical significance of the measured odd ratio is assessed by a special χ^2 formula. Deviations from Hardy-Weinberg equilibrium were investigated for all polymorphisms using the $\chi 2$ statistic, with expected frequencies derived from allele frequencies. An estimate was considered statistically significant if its P value was less than an α level of significance of 0.05. Adjustment for the hypothetically proposed increase in the alpha level of significance in case of multiple comparisons of different genotypes was done by multiplying the P value by the number of comparisons.

Results:

Twenty control subjects and 69 individuals suffering from asthma were recruited and genotyped for *ADAM33-V4* polymorphism. Table 1 and 2 show the case-control difference in mean of age and gender distribution. Genotyping analyses revealed V4 SNP in the 3′ region of *ADAM33*, figure (1), being distributed in Hardy-Weinberg equilibrium.

Table (1): The case-control difference in mean age.

	Healthy controls	Cases (Asthma)	P
Age (years)			**0.83[NS]**
Range	**(18 - 69)**	**(18 - 70)**	
Mean	**35.3**	**34.4**	
SD	**17.3**	**16**	
SE	**3.86**	**1.92**	
N	**20**	**69**	

❖ **NS= No Significant, SD= Standard Deviation, SE= Standard Error, N= Number**

Table (2) : The case-control difference in gender distribution.

Case-control comparison					
	Healthy controls (n=20)		Cases (Asthma) (n=69)		P
Gender	N	%	N	%	0.23[NS]
Female	11	55.0	48	69.6	
Male	9	45.0	21	30.4	
Total	20	100.0	69	100.0	

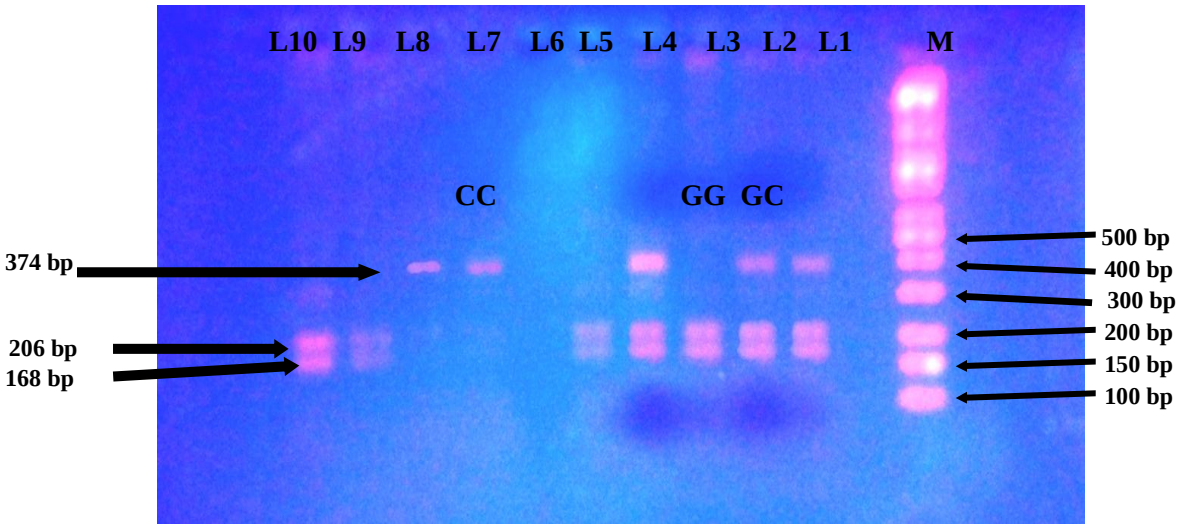

Figure (1): Ethidium bromide-stained agarose gel of PCR – RFLP amplified 374 bp of *ADAM33* gene for study groups. Lane (M): DNA molecular size marker (KAPA Universal Ladder), Lane 3,5,9,10= GG genotype (168/206 bp), Lane 7,8=CC genotype (374 bp), Lane 1,2,4=GC genotype (168/206/374 bp)

Distributions of genotypes and alleles in cases and controls groups.

Table (3) showed the increase risk for asthma was observed for the homozygous mutant genotype (GG), mutant allele (G) and heterozygous genotype (CG) of the SNP rs2787094 and demonstrated the G allele is higher frequency in asthmatic patients than C allele but this convert in controls.

Table (3):Distribution of genotypes and alleles of *ADAM33/V4* in case- control.

SNP		Case-control comparison									
		Healthy controls (n=20)		Cases (Asthma) (n=69)							
rs2787094 (C>G)	Genotype	N	%	N	%	P	OR	95% CI OR	Adjusted P	EF	PF
	GG	1	5	31	45	< 0.001	10.6	(2.2 to 186.6)	0.001	0.41	**
	CG	4	20	29	42	0.023	4.5	(0.9 to 33.9)	0.122	0.33	**
	CC	15	75	9	13	0.451	0.06	(2 to 0.11)	0.451[NS]	**	0.88
	Allele										
	G	6	15	91	65.9	< 0.001	10.2	(3 to 57.9)	0.002	0.59	**
	C	34	85	47	34.1	0.6[NS]	0.1	(0.12 to 2)	>0.05	**	0.75

❖ **OR=odd ratio, p= p value, EF= etiology fraction, PF=preventive fraction**

***ADAM33* polymorphism in patients with and without family history of asthma**.

To test a possible difference in the genotype /allele distribution pattern in patients with or without family history of asthma, Bar Charts were performed (figures 2 and 3). The study found that the homozygous mutant genotype (GG) and mutant allele (G) of SNP rs2787094/V4 was significantly associated with patients who had a family history of asthma as compared with patients without family history of asthma, whereas heterozygous (GC), normal homozygous genotypes (CC) and (C) alleles of these SNP associated with asthmatic patients without family history.

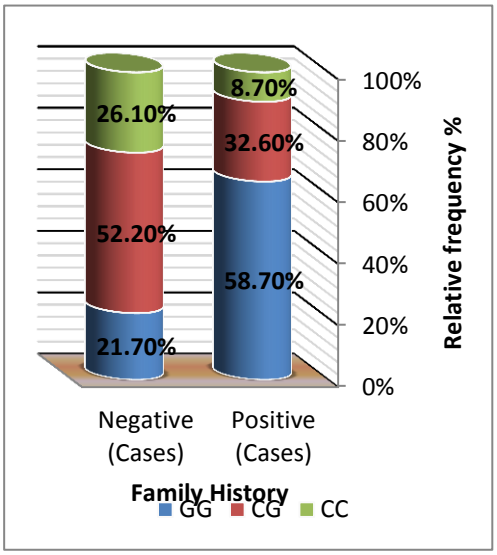

Figure (2): Bar Chart show genotypes distribution in asthmatic patients with and without family history.

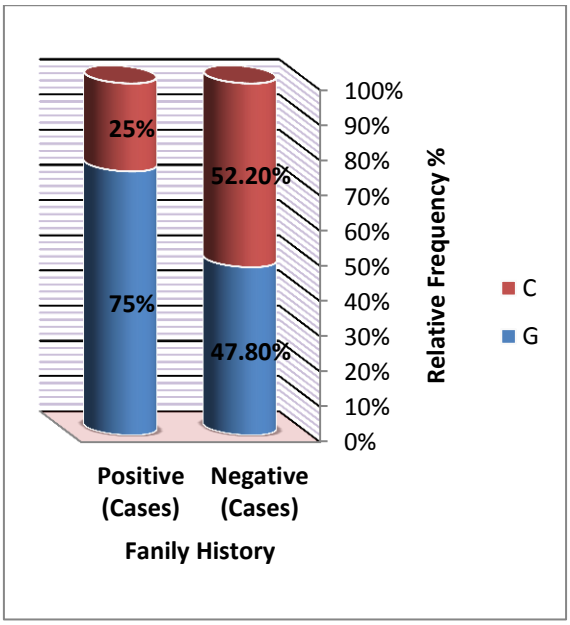

Figure (3): Bar Chart show alleles distribution in asthmatic patients with and without family history

Geographic differences of the observed genotype and allele frequencies of the *ADAM33*-V4 G/C polymorphism

A comparison of observed genotype and allele frequencies of the *ADAM33-V4 G/C* SNP in this study with the other published studies investigating the association of this polymorphism and asthma worldwide, the present study found that the frequency of the minor G allele containing GC and GG genotypes in asthmatic patients were different as shown in table (4).

Table (4): Comparison of *ADAM33* –V4 frequencies in different case-control populations

Study population	Genotypes or Alleles	Cases No. (%)	Controls No. (%)	P -value	References
USA and UK	C	109 (83.6)	166 (76.7)	0.03	Van Eerdewegh *et al.* (2002)
	G	21 (16.4)	51 (23.3)		
Mexican	C	224 (84.7)	154(82.8)	0.537	Lind *et al.*(2003)
	G	41 (15.3)	32(17.2)		
German	CC	267 (58.6)	474 (62.5)	>0.05	Schedel *et al.* (2006)
	CG	163 (35.7)	249 (32.9)		
	GG	26 (5.7)	35 (4.6)		
Thai	CC	87 (43.5)	40 (40)	>0.05	Thongngarm *et al.*(2008)
	CG	94 (47)	47 (47)		
	GG	19 (9.5)	13 (13)		
Chinese Han	CC	49(27.07)	113(74.84)	<0.001	Su *et al.* (2008)
	GC	78(43.09)	32(21.19)		

	GG	54(29.84)	6(3.97)		
Indian	CC	17 (7.9)	47 (18.6)	<0.001	Tripathi P *et* al. (2011)
	CG	70 (40)	133 (52.6)		
	GG	88 (50.3)	73 (28.6)		
	C	104 (29.7)	227 (44.9)	<0.001	
	G	246 (70.3)	279 (55.1)		
Iraq	CC	6 (13)	15 (75)	<0.001	Current study (2013)
	CG	29 (42)	4 (20)		
	GG	31 (45)	1 (5)		
	C	47 (34.1)	34 (85)	<0.001	
	G	91 (65.9)	6 (15)		

Discussion:

Asthma is a common, chronic disease of airway inflammation that manifests with recurrent episodes of coughing, breathlessness, wheezing, and chest tightness. These episodes are associated with airflow obstruction that is at least partially reversible. In this case–control study, we found (tables 1 and 2) the highest frequency of asthma among adult patients particularly from age 20-35 years old (31.9%), followed by the age group of < 20 years old (27.5%**)** this differences may be due to high life activities during this range as works and exercise which lead to increase sensitivity to allergens, also hormonal changes in female (as during pregnancy and menstrual) may be increase frequency of asthma **(14,21)**. Table (3) showed the significant association of V4 SNP of spanning *ADAM33* with the adult-onset asthma in our population. The study found that the homozygous mutant genotype, heterozygous genotype and mutant allele frequencies of SNP rs2787094 had significant association with asthma, this may act together for increase the risk of asthma **(14)**. C allele might serve as protective factors for the disease **(15)**. Furthermore, figures 2 and 3 showed that *ADAM33* was inheritable gene and strongly associated with inheritance of the asthmatic families **(16)**. the G allele and homozygous mutant GG genotype act as risk factors in patients with positive family history. current results extend the previous findings on possible role of *ADAM33* in genetic susceptibility to asthma in diverse populations **(17)**. Table (4) observed lack of association for *ADAM33* gene in asthma have been reported in populations from Mexican, German and Thai. Intriguingly, the V4 SNP has significant association with the adult-onset asthma in other population as Indian, US and UK **(11,15,17)**.

Thus, the present study adds to the growing list of population studies where the *ADAM33*-V4C/G may play a role in the susceptibility to asthma. The studies also highlight the fact that no single SNP is associated with asthma in all the populations studied **(17)**. Firstly, this may be due to the compound effects of multiple alleles, multiple genes and environmental factors since asthma is a multi-factorial disorder. The genetic diversity of diversity reflected in differences in the occurrence of polymorphisms and their allelic frequencies. For instance, the allelic distributions of the SNP V4 in our population are different from other populations genetics diversity among these populations and genetic differences among subtypes of asthma, such as pediatric, adult, allergic/non-allergic also may play role **(18)**. Secondly, this could be due to the difference in the exposure to allergens, which could vary from one geographical region to the other and their

interactions with the genetic factor(s) could vary **(19)**. Thirdly, the linkage disequilibrium patterns that exist between the identified V4 and the undetected causative defect in the gene could differ from one population to the other. Thus through understanding the genetic factors and their interactions with the environmental factors for each population would aid in developing effective predictive markers for the prevention and/or management of multifactorial diseases like asthma **(20,21)**.

Conclusion:

Although *ADAM33*-V4 polymorphism not associated with asthma in many population, this study confirmed significant correlation between *ADAM33*-V4 and asthma.

Recommendations:

1. Study the association between other SNPs of *ADAM33* gene and bronchial asthma.
2. Pharmacogenomic study for decrease the effects of large numbers of genetic factors that contribute to the heterogeneity of asthma.

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