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Association of Adam33 Gene SNPS with Asthma in a Local Pakistani Population

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ABSTRACT

Asthma is a chronic inflammatory disorder of lungs and airways regardless of the development in disease treatment the number of affected individuals is increasing day by day all around the world. Though largely considered environmental, asthma is an interactive disease and is caused by interaction of both environmental and genetic factors. A number of genes are found to be associated with asthma but recently discovered *ADAM33* is now considered candidate gene for asthma. Two *ADAM33* genetic variants rs2787094 C/G and rs3918936 A/G were genotyped using PCR-RFLP in a case-control study design. The association of genotyped SNPs was statistically explored for asthma. Gender and age specific disease trends were also checked. Our results clearly showed a strong association between asthma susceptibility and *ADAM33* rs2787094 C/G polymorphism while *ADAM33* rs3918396 lacked significance in association. Both *ADAM33* SNPs lacked age or gender based disease association. We report strong association of *ADAM33* rs2787094 C/G polymorphism with asthma in local Pakistani population irrespective of age and gender. While a lack of disease association with *ADAM33* rs3918396 SNP with our population is in accordance with other Asian populations.

Keywords: Genetic marker, Polymorphism, Inflammatory Disorder, Disease Association

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INTRODUCTION

Asthma is a complex chronic inflammatory disorder with multiple phenotypes characterized by cough, wheezing, bronchial arrest and chest congestion. Disease condition manifests due to multiple factors including genetic and environmental factors. The genetic factors alone can contribute up to 79% whereas the role of environmental factors is only 21 %. Prevalence of asthma is increasing to an alarming rate and becoming an epidemic in developed countries with approximately 300 million people worldwide suffering from disease. Asthma is regarded as interplay of environmental and genetic risk factors. A number of asthma candidate genes along with polymorphic markers have been identified in various ethnic groups worldwide *ADAM33*, a member of ADAM gene family, is one of the recently studied candidate gene for asthma susceptibility. The gene is located on chromosome 20p13 with multiple domains involved in different cellular processes such as activation, proteolysis, adhesion, fusion, and intracellular signaling. The protein has two isoforms, α and β. The α-form is found abundantly in airway fibroblasts, myofibroblasts, and smooth muscles cells. Any changes in the activity of *ADAM33* suggest abnormalities in the function of airways smooth muscle cells and fibroblasts. Several single nucleotide polymorphisms (SNPs) have been identified in ADAM33 gene and genetic studies have confirmed associations of these variants with asthma and structural changes in airways[1]. Association of asthma with *ADAM33* SNPs has a generalized pattern among many genotyped world populations and no single disease susceptibility SNP has yet been reported/ discovered. Most of the genetic association studies for asthma and ADAM33 gene polymorphisms have been reported on Caucasian and Chinese populations. The present case control study was conducted to assess the association of specific *ADAM33* variants (V4 rs2787094 and S1 rs3918396) with asthma in local Pakistani population.

MATERIALS AND METHODS

Study subjects

Diagnosed asthma patients (N=298) along with healthy controls (N=204) were recruited from general hospitals located in Islamabad and Rawalpindi, Pakistan. Patients were selected on the basis of international diagnostic criteria; Clinical examination, peak expiratory flow rate and Spirometry. Prior to blood sampling, written informed consent was obtained. The study was approved by the Institutional Ethical Committee and was in compliance with Helsinki Declaration.

DNA extraction and genotyping

Genomic DNA was extracted from 5ml whole blood using modified protocol of DNA extraction [5]. Two SNP's of ADAM33 gene, V4 (rs2787094) and S1 (rs3918396) were selected for genotyping. These SNPs had already been studied in different World populations and have shown association with asthma. Both SNPs were genotyped based on PCR-RFLP protocols.

Statistical analysis

Due to missing genotype counts, there were 295 cases and 204 controls for rs2787094 and 298 cases and 204 controls for rs3918396. Genotype and allele frequencies were computed calculated and Hardy Weinberg Equilibrium computed. Pearson chi square test at 5% significance level was applied to confirm if genotype and allele frequencies among cases and controls are in Hardy Weinberg proportions for the screened polymorphism(s). The sex (Male/Female) and age based (<40 years and >40 years) comparisons among studied population were calculated using Student's t-test. Association among rs2787094 SNP genotypes and disease were estimated using Logistic Regression method in an age and sex adjusted data. Odds ratios (OR) and Confidence Intervals (CI) at 95% significance level were computed. All statistical analyses were performed using SPSS (Version 16.0; SPSS, Inc). Significance of analyses was considered with $\rho \leq 0.05$.

RESULTS AND DISCUSSION

The sex and age based distributions of total study population are provided in Table 1. Among cases and controls the ratios of male (44.3% and 49.3%) to female (55.7% and 50.7%) did not deviate much. As for the two age groups, in younger age group (>40) 51.2% subjects were diseased and 48.8% were controls, while 77.0% of the asthma cases were found in the subjects >40 age group. The genotype and allele frequencies for *ADAM33* SNPs rs2787094 and rs3918396 among cases and controls are presented in Table 2. Of two SNPs, rs2787094 was in HWE while rs3918396 showed a clear deviation therefore only rs2787094 was analyzed for disease association. For rs2787094 SNP, genotype frequencies of homozygous CC allele was raised (p=0.0015) in asthma cases as compared to control population while homozygous GG genotype was significantly among controls as compared to cases (p=0.0002). However heterozygous genotype frequencies did not differ much among cases and controls (p=0.408). The frequencies for C and G alleles are provided in Table 2 showing significant differences among cases and controls. The frequency of C allele was significantly raised in cases (<0.0001) as compared to controls while G allele frequency lacked any

significant distribution among cases and controls (0.13) in our studied population. In case of rs3918396 SNP, homozygous GG and heterozygous AG genotypes were significantly high in asthma cases as compared to controls ($p<0.0001$) while homozygous AA genotype was significantly raised in control population ($p<0.0001$). The A allele frequency of rs3918396 SNP was significantly high in control subjects while G allele was high in asthma cases (<0.0001). The results for genetic association of rs2787094 SNP with asthma are presented in Table 3. As shown in Table 3, our population seemed to follow dominant genetic model with regards to C allele in an age and sex adjusted data set. We found significant association of rs2787094 SNP with disease in our studied population ($p<0.0001$). The C allele carriers were at higher risk of disease (OR= 2.08 CI=1.45-2.99) as compared to healthy controls. Our data showed strong role of the age of study participants towards disease susceptibility even after adjustment. There was highly significant high risk of asthma in cases >40 years of age (OR=12.94 CI=4.31-38.85 $p<0.0001$) as compared to <40 years of age group. Our data lacked effect of sex towards disease risk (OR=1.29 CI=0.87-1.9 $p=0.19$). The findings of present study are in agreement with previous reports carried out in other world populations to establish association between *ADAM33* rs2787094 SNP's C allele and asthma. Strong association of rs2787094 SNP with asthma ($p=0.0001$) have been reported in Han Chinese and South Indian populations. A case control study carried out in US/UK combined and UK only population also supports findings of present study showing significantly strong association ($p=0.03$) of C/G polymorphism with asthma along with C allele frequencies of 83.6% in US/UK combined and 83.7% in UK only populations. Likewise case control studies carried out in ethnically diverse populations including; Dutch, African American, US white, and US Hispanic populations also report rs2787094 SNP association with disease. However some other disease association studies reported in family-based as well as case-control studies in Mexican/Puerto Rican, Colombian, Egyptian population [10], South Indian [11] and Australian [12] populations did not find association of rs2787094 SNP with asthma. Thus in the light of previous findings, results of present study clearly indicate a highly diverse ethnic specific association pattern of *ADAM33* SNPs like rs2787094 with asthma. Due to ethnic diversity no single SNP is universally associated with asthma susceptibility, it is possible that a particular set of SNPs may be associated with increased risk of asthma and other respiratory disorders in Caucasians and African and different subset is involved in our study population.

Table 1: Sex and Age Groups based Population Characteristics

	Cases No. (%)	Controls No. (%)
Sex Groups (M/F)		
Male	131 (44.3)	102 (49.3)
Female	165 (55.7)	105 (50.7)
Age Groups (Years)		
<40	172 (51.2%)	164 (48.8%)
>40	134 (77.0%)	40 (23.0%)

Distribution of study subjects in sex and age based groups under case-control model

Table 2: Genotype and Allele Frequency Distribution of ADAM33 SNPs among Cases and Controls

SNP ID	Genotype Frequencies No. (%)		
	CC	**GG**	**GC**
rs2787094			
Cases	82 (0.278)	82 (0.278)	131 (0.444)
Controls	32 (0.157)	89 (0.436)	83 (0.407)
	Allele Frequencies No. (%)		**ρ-value**
	C	**G**	
Cases	295 (0.50)	295 (0.50)	<0.0001
Controls	147 (0.36)	261 (0.64)	0.13
	Genotype Frequencies No. (%)		
rs3918396			
	AA	**GG**	**AG**
Cases	89 (0.294)	131 (0.432)	83 (0.274)
Controls	169 (0.845)	4 (0.2)	27 (0.135)
	Allele Frequencies No. (%)		**ρ-value**
	A	**G**	
Cases	261 (0.431)	345 (0.569)	<0.0001
Controls	365 (0.913)	35 (0.088)	<0.0001

Comparison of genotypes and allele frequencies of ADAM 33 SNPs among cases and controls. A p value ≤0.05 shows significant differences among compared groups

Table 3: Association of rs2787094 SNP with Asthma

Variables	OR (CI at 95 %)	p-value
Age (Years)		
<40	1	<0.0001
>40	12.94 (4.31-38.85)	
Sex		
Male	1	0.19
Female	1.29 (0.87-1.9)	
(Dominant) G/G	1	<0.0001
C/G-C/C	2.08 (1.45-2.99)	

CONCLUSION

Our findings suggest that ADAM33 gene may be involved in the susceptibility to asthma in local Pakistani population. Older subjects are at higher disease risk irrespective of sex. The C allele of rs2787094 SNP was found as risk allele with higher frequency in cases as well as showing increased asthma risk in our study population. It must be noted that our study included limited number of individuals which only represent small group of entire Pakistani population, not a true representatives of whole population.

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