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Haplotype analysis of *ADAM33* polymorphisms in asthma: A pilot study

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***Background & objectives*: *ADAM33* is implicated as a potentially strong candidate gene for asthma and bronchial hyper-responsiveness. Many polymorphisms of *ADAM33* have been studied along with ADAM33 expression in various cells of the lungs. Haplotype analysis also showed association with asthma in different populations across the world. Therefore, the aim of this study was to perform a comprehensive screening of *ADAM33* polymorphisms in adult patients with asthma.**

***Methods*: Thirty five polymorphisms of *ADAM33* were genotyped in 55 patients with asthma and 53 controls. The association of single nucleotide polymorphisms (SNPs) and haplotypes with phenotypes of asthma was analysed.**

***Results*: The genotype, minor allele frequency, odds ratio and Hardy–Weinberg equilibrium did not show any significant difference among cases and controls. No association was found between SNPs of *ADAM33* with the severity of asthma. Correlation analysis of *ADAM33* SNPs to the phenotypes, based on clinical variables and allergen sensitization, did not show significant difference. Haplotype analysis showed that rs2280090 and rs2280091 were associated with asthma in the patient group.**

***Interpretation & conclusions*: Haplotype analysis showed an association of the two SNP variations with asthma. These SNPs lead to amino acid change and are prone to phosphorylation, which may affect expression levels and protein function of ADAM33 and asthma susceptibility.**

Key words ADAM33 - asthma - haplotype - polymorphisms - rs2280090 - rs2280091 - SNP

Asthma is associated with multiple single nucleotide polymorphisms (SNPs) of several genes; among them, *ADAM33* is considered as a potentially significant asthma candidate gene. *ADAM33* is one of the few genes that have been consistently found to be associated with asthma and bronchial hyper-responsiveness, and the findings have been reproduced in more than six independent populations; Caucasian, African-American, Hispanic, Japan, China, Korean and Jordan[1-3]. *ADAM33* has been shown to

have a higher expression in epithelial cells and smooth muscle cells of airways among asthmatics compared to controls. It has been identified as a potential therapeutic target for asthma as it enables airway inflammatory and remodelling effects after allergen exposure[4,5]. Every individual's genotype entails multiple closely linked SNPs, which can be referred to as haplotypes. Haplotypes are analyzed mainly for two reasons; primarily, haplotype alleles may exist in closer linkage disequilibrium with causal variant compared to single measured SNP and the other reason being that the haplotypes can be causal variants of significance by themselves[6]. The haplotype analysis can be considered to narrow down the position of disease susceptible loci, complex disease gene mapping and genome-wide association studies and to reconstruct the histories of human population[7,8]. Common haplotype frequencies can be evaluated based on the known marker phenotypes in unrelated individuals of a population[9].

ADAM33 is important in asthma and is one of the few associations that have been successfully reproduced in follow up studies, including the haplotype analysis in different populations across the world. Over 55 different SNPs of *ADAM33* have been reported in the literature, and the majority of these have been associated with asthma[10]. The present pilot study was carried out to comprehensively screen 35 *ADAM33* polymorphisms that have been known to be associated with asthma in patients with asthma and healthy individuals.

Material & Methods

Consecutive patients with asthma (n=55) visiting the JSS Hospital, Mysuru, India, from July 2012 to January 2013 were included in the study. The age- and gender-matched controls were selected from the general population from the Burden of Obstructive Lung Disease (BOLD) cohort in urban Mysuru[11]. Both patients and normal controls underwent spirometry, pre- and post-bronchodilator, satisfying the American Thoracic Society standards[12]. The diagnosis of asthma was based according to the Global Initiative for Asthma (GINA) guidelines having a reversibility of more than 12 per cent and 200 ml on post-bronchodilator spirometry[13]. Asthma patients were classified according to severity based on the GINA-2012 guidelines as mild-, moderate- and severe-persistent asthma (*https://ginasthma.org/wp-content/uploads/2019/01/2012-GINA.pdf*). All the asthmatics underwent skin prick testing to confirm atopy. The patient was said to be atopic if he/she was sensitized to any one of the allergens tested. A wheal size of more than 3 mm of saline control was considered a positive skin prick test. Three millilitres of venous blood was collected into BD Vacutainer® Plus Plastic K_2 EDTA tubes (USA). Wizard Genomic DNA isolation kit (Promega, USA) was used to isolate genomic DNA from peripheral blood leucocytes.

The patients below 18 yr were excluded from the study. Further, the patients with any other respiratory diseases were also excluded. The Institutional Human Ethical Committee of University of Mysore approved the present study (IHEC-UOM No.79 Ph.D/2012-13). Written informed consent was taken from all the patients/participants of the study.

Single nucleotide polymorphism (SNP) genotyping using MassARRAY analysis: SNP genotyping in the samples was performed using the Sequenom MassARRAY platform using MALDI-TOF mass spectrometry in Xcelris Genomics Company, Ahmedabad, India, wherein Sequenom-iPLEXR Gold SNP genotyping-platform (Agena Bioscience™, CA, USA) along with Spectro CHIP was used for the MassARRAY method and the analysis was done with MALDI-TOF mass spectrometry.

Analysis of SNPs of ADAM33 gene: *ADAM33* consists of 22 exons and 21 introns encoding 813 amino acids. There are more than 340 SNPs identified in this gene[14]. This study was performed on 35 SNPs of *ADAM33* selected based on previous studies[15,16] as well as from the databases (*https://www.ncbi.nlm.nih.gov/snp/* and *https://www.snpedia.com/index.php/SNPedia*). The SNPs selected and their positions on the genome along with the type of SNPs, primers used for *ADAM33* SNP genotyping alongside the mass of unextended (E1) and extended (E2) primers in Daltons are listed in Table I.

The "PED" format file with case-control values paired to the locus information file was considered for the haplotype analysis using Haploview tool (*https://www.broadinstitute.org/haploview/haploview*) which identified the association with T-int value. The association test was performed using the case/control possibility. The pairwise comparison of markers >500 kb distance was ignored followed by excluding individuals with >50 per cent missing genotypes. There were no individuals with missing genotype in our study dataset. The haplotype blocks were created with >1.0 per cent linked haplotype, and >10.0 per cent haplotype blocks were highlighted with the thick lines for association identification.

Table I. List of 35 single nucleotide polymorphisms (SNPs) of *ADAM33* used for genotyping of *ADAM33* variants in healthy controls and asthma patients

SNP rs ID	Position in bp	Type of variant	UEP sequence	Allele (E1/E2)	Mass of UEP/E1/E2 (Dalton)
rs2280091	3669587	Non-synonymous	GGGCGGCGTTCACCCCA	G/A	5172.4/5419.5/5499.5
rs2787094	3668514	3'UTR	GTCCACACTCCCCTG	G/C	4448.9/4696.1/4736.1
rs3918391	3675071	Non-synonymous	CCAGGATACATAGAAACCCAC	C/T	6377.2/6624.4/6704.3
rs3918396	3671118	Non-synonymous	GGCCATGCTCCTCAGC	A/G	5107.3/5378.5/5394.5
rs677044	3668784	3'UTR	CCAGCCCTCAGGAACTTCTA	G/A	6021.9/6293.1/6309.1
rs2853209	3670825	Intronic	TGGCCTCCCAGTCAAGCG	T/A	5764.7/6036/6091.8
rs3918392	3674572	Non-synonymous	GCTCACCTGGAAAGGA	A/G	6176/6447.2/6463.2
rs137919189	3668971	Non-synonymous	GCTACCTCTCACCAGA	A/G	5394.5/5665.7/5681.7
rs41462450	3671022	Non-synonymous	TGGGGCTGCAGAAGG	G/T	5340.5/5627.7/5667.6
rs6084434	3674821	Synonymous	ACTACCAAGGGCGAGTAA	T/C	5541.6/5812.8/5828.8
rs3918394	3672848	Non-synonymous	CCCCCCTTGCGGAAGAAG	T/C	6970.5/7241.7/7257.7
rs3918401	3668993	Non-synonymous	TCCTCATTCTCAGCAGATCA	A/T	6341.1/6612.3/6668.2
rs2787093	3667815	Downstream	GCAGGCCGAGCCTAG	T/C	5470.6/5741.8/5757.8
rs511898	3674438	Intronic	TCGAGGCCTGTGAATTCC	C/T	6775.4/7022.6/7102.5
rs2485700	3674346	Intronic	CTTCTGGGAGCTGGG	C/T	6140/6411.2/6427.2
rs2271510	3673503	Intronic	CGAGTGGTCCTGGGG	A/G	5595.6/5866.8/5882.8
rs2485699	3674341	Intronic	TGGGAGCTGGGATTGG	C/A	5033.3/5320.5/5360.4
rs574174	3670047	Intronic	AATGACAAGGCCTTGGG	T/C	5259.4/5530.6/5546.6
rs12479696	3669310	Non-synonymous	TGCCAGCAGTCTCGC	G/A	5998.9/6246.1/6326
rs615436	3672188	Non-synonymous	GGGGCAGTGGCTACT	G/A	4649/4896.2/4976.1
rs17513846	3669893	Intronic	TCCTCCAGGCTCTGA	C/A	4503.9/4751.1/4775.1
rs614971	3668203	3'UTR	ATCACCAGAGGCCAG	C/G	4571/4818.2/4858.2
rs41419248	3673394	Synonymous	GGCTGCTGCGTGGAGGCT	G/T	6214/6501.2/6541.1
rs41534847	3673835	Synonymous	GGACCGCAGCCGCGTCA	T/C	5181.4/5428.6/5508.5
rs41467948	3675034	Non-synonymous	TGGTGCTGGCCCCCA	G/A	5451.5/5722.7/5738.7
rs41382144	3672615	Non-synonymous	GTGAGCAAAGCAGCAGAG	G/C	5935.9/6183.1/6223.1
rs528557	3671095	Synonymous	TGCCTCTGCTCCCAGG	G/C	4809.1/5056.3/5096.3
rs17548872	3669884	Intronic	CTCTCTAGTCCTAACATTTCCTC	C/G	6860.5/7107.7/7147.7
rs41492952	3670152	Intronic	GGCGTGTCAGCACGGA	C/G	6577.3/6824.4/6864.5
rs2280090	3669558	Non-synonymous	CACAGCCACTGGACAG	G/A	5486.6/5733.8/5813.7
rs3918400	3668816	3'UTR	CTTCCCCGAGTGGAGCTT	T/C	6414.2/6661.3/6741.3
rs6084435	3682089	5'UTR	ACCCGTGCCCGGTGC	A/G	5701.7/5972.9/5988.9
rs11905233	3668529	3'UTR	CCCTATGGTTCGACTGA	T/C	5161.4/5432.6/5448.6
rs3746631	3668493	3'UTR	GGCTGGCCTCTGCAA	T/C	4569/4840.2/4856.2
rs517155	3668230	3'UTR	TGATCCTCCTACCCC	G/A	4423.9/4695.1/4711.1

Source: Refs 15,16
UTR, untranslated region; SNPs, single nucleotide polymorphisms; UEP, unextended primer

Effect of SNP variation: The possible effect of an amino acid change on the structure and function of a human protein can be predicted by the tool, PolyPhen-2 (Polymorphism Phenotyping V2; *http://genetics.bwh.harvard.edu/pph2/*). PolyPhen-2 uses eight sequence-based and three structure-based predictive features, which are selected automatically by an iterative greedy algorithm. The majority of

these features involve comparison of a property of the wild-type (normal) allele and the corresponding property of the mutant (derived, disease-causing) allele, which together define an amino acid replacement[17].

Statistical analysis: SNPs of *ADAM33* were related with asthma using Hardy–Weinberg equilibrium, Chi-square test, odds ratio, ANOVA and Student's *t* test to understand the association of *ADAM33* variations with asthma. These association tests between clinical variables and SNPs were tested using SPSS software V.19 (SPSS, Inc., Chicago, USA). Haploview software V.4.0 (Broad Institute, Cambridge, MA, USA) was used to assess the SNP haplotype and disease association.

Results

The demographic characteristics of the population studied are presented in Table II. Genotype to phenotype, minor allele frequency, odds ratio and Hardy–Weinberg equilibrium calculated did not show significant difference among cases and controls (data not shown). Thirty five polymorphisms of *ADAM33* and their haplotypes were analysed for their association with asthma in 19 mild, 21 moderate and 15 severe cases. Table III represents the pulmonary function test values for both patients and controls. There was no association with any of the SNPs in mild, moderate or severe asthma as compared to controls. A comparison of the genotype distributions of *ADAM33* SNPs among atopic and non-atopic patients showed an insignificant difference. Correlation analysis of *ADAM33* SNPs using Chi square statistics to the clinical variables did not show positive correlation with the allergen sensitization, either with the number of allergen sensitization or with the severity of sensitization.

Table II. General characteristics of the study population

Variables	Controls n=53	Patients n=55
Age (yr)		
≤40	35 (66.03)	31 (56.36)
>40	18 (33.96)	24 (43.63)
Gender		
Male	22 (41.50)	20 (36.36)
Female	31 (58.49)	35 (63.63)
Family history of asthma		
Yes	0	24 (43.63)
No	53 (100)	31 (56.36)
Asthma duration (yr)		
<1	-	4 (7.27)
1-5	-	20 (36.36)
>5	-	31 (56.36)
Severity		
Mild persistent	-	19 (34.54)
Moderate persistent	-	21 (38.18)
Severe persistent	-	15 (27.27)
Atopic patients	-	52 (94.54)
Non-atopic patients	-	3 (5.45)

Values in parenthesis are percentages

Haplotype analysis: Haplotype analysis showed that SNPs rs2280090 and rs2280091 were associated with asthma in the patient group (Table IV). The association of haplotype with asthma was analyzed using Haploview software similar to other studies[18-20]. The haplotype with AA alleles was found to be significant (P=0.037) (Table V). The SNP location was 29 bp apart from each other and the LOD score was 20.48 followed by D --- 1. The r^2 value was 0.75 and the distance between these SNPs was 29 bp. The Haploview software uses its own statistical value called T-int, which was found to be 105.54 (Table V and Fig. 1).

PolyPhen-2 analysis of significantly associated variations showed the effect 'benign' for both rs2280090 and rs2280091 SNPs individually (Fig. 2).

Discussion

Asthma is a heterogeneous, chronic lung disease that is caused by interplay of genes and environment in an individual. *ADAM33* is one of the positionally cloned asthma-associated genes found to be associated with many pathological features of asthma, including goblet cell hyperplasia, subepithelial fibrosis, collagen deposition, mucosal gland hyperplasia, smooth muscle hypertrophy, changes in the extracellular matrix and inflammation[4,21,22]. In the present study the genotype to phenotype, minor allele frequency, and odds ratios did not show significant difference among cases and controls with SNPs. On haplotype analysis, an association of two SNPs rs2280090 and rs2280091 was observed with asthma with a strong linkage disequilibrium value.

Many SNPs associated with asthma have not been found to be reproducible in different ethnic populations such as Puerto Rican and Mexican[23]. *ADAM33* SNPs have been found to be associated with asthma susceptibility in various ethnic populations, though

Table III. Pulmonary function test results of controls and patients of the study population

Pulmonary function test parameters	Controls (pre-bronchodilator) (n=53)	Patients (pre-bronchodilator) (n=55)	Patients (post-bronchodilator) (n=55)
FVC (%)	88.44±0.511	70.63±0.82	78.3±0.88
FEV1 (%)	88.84±0.47	69.12±0.89	78.81±0.72
FEV1/FVC (%)	104.23±0.35	99.54±0.62	106.83±0.53
PEF (l/sec)	92.81±0.95	74±1.0	84.56±0.90

Values are mean±SE. FEV1, forced expiratory volume in one second; FVC, forced vital capacity; PEF, peak expiratory flow

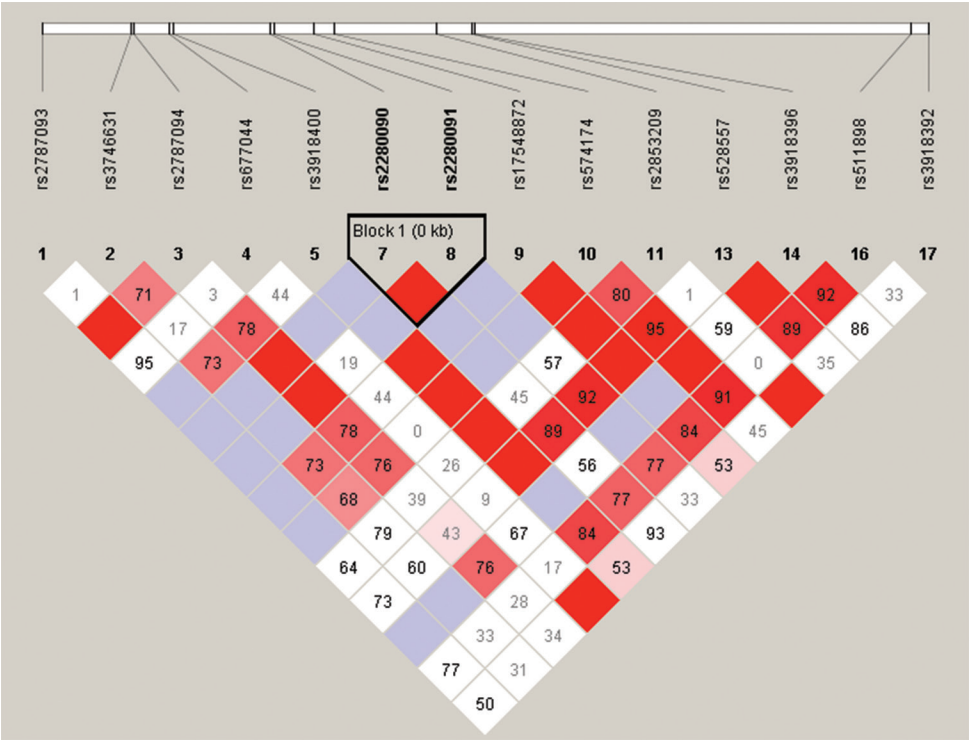

Fig. 1. Haplotype blocks showing association between asthma and *ADAM33* polymorphisms rs2280090 and rs2280091. The two SNPs are 29 bp apart from each other with a LOD score of 20.48.

with different frequencies, and are one of the most reproducible variables with odds ratio 1.67 to 4.34 (*e.g.*, US White and US Hispanic, Black Americans, UK, Europeans and Asians)[24].

The study on *ADAM33* polymorphisms was carried out as continuation of studies in genetic polymorphisms in asthma by the Mysore asthma genetics group[2,25,26]. No association was found in our study between *ADAM33* SNPs and asthma phenotypes such as asthma severity and atopy. Our results were similar to other studies conducted in Australian and German populations[1,27]. On the other hand, in a study from north India[28] one of the SNPs of *ADAM33* was found associated with the mild intermittent subgroup of asthma[28].

On haplotype analysis, two polymorphisms, rs2280090 and rs2280091, were observed to be associated with asthma among 35 SNPs, with the T-int value of 105.54. However, Haploview software measures the completeness of information represented by a set of markers in a region and denoted as T-int. The value of T-int >100 is considered as significantly associated as per the Haploview tool. The result supported another study where haplotypes were found to be associated with asthma[18]; further, these SNPs were associated with an increased predisposition to asthma[29], but no such association was found in other studies[30,31]. In another study, rs2853209 SNP was found to be associated with asthma along with T1 (rs2280091) and F+1 SNPs of *ADAM33*[32].

Table IV. Haplotype analysis of *ADAM33* single nucleotide polymorphisms among patients and controls

L1	L2	D'	LOD	r^2	CI-low	CI-hi	Dist	T-int
rs2787093	rs3746631	0.016	0	0	−0.01	0.28	678	6.91
rs2787093	rs2787094	1	5.28	0.213	0.7	1	699	-
rs2787093	rs677044	0.955	0.28	0.02	0.06	0.97	969	-
rs2787093	rs3918400	1	0.59	0.007	0.09	0.99	1001	-
rs2787093	rs2280090	1	0.76	0.017	0.12	0.99	1743	-
rs2787093	rs2280091	1	0.44	0.012	0.07	0.98	1772	-
rs2787093	rs17548872	1	0.59	0.007	0.09	0.99	2069	-
rs2787093	rs574174	1	1.14	0.022	0.18	0.99	2232	-
rs2787093	rs2853209	0.646	1	0.052	0.15	0.87	3010	-
rs2787093	rs528557	0.733	0.94	0.025	0.16	0.92	3280	-
rs2787093	rs3918396	1	0.4	0.012	0.07	0.98	3303	-
rs2787093	rs511898	0.776	1.26	0.029	0.22	0.94	6623	-
rs2787093	rs3918392	0.5	0.03	0.002	0.04	0.96	6757	-
rs3746631	rs2787094	0.711	2.67	0.13	0.37	0.88	21	21.21
rs3746631	rs677044	0.179	0.25	0.011	0.02	0.46	291	-
rs3746631	rs3918400	0.734	8.66	0.478	0.53	0.86	323	-
rs3746631	rs2280090	1	1.33	0.02	0.22	1	1065	-
rs3746631	rs2280091	1	0.95	0.015	0.14	0.99	1094	-
rs3746631	rs17548872	0.734	8.66	0.478	0.53	0.86	1391	-
rs3746631	rs574174	0.68	3.49	0.165	0.39	0.85	1554	-
rs3746631	rs2853209	0.79	1.44	0.038	0.25	0.94	2332	-
rs3746631	rs528557	0.608	1.26	0.061	0.18	0.83	2602	-
rs3746631	rs3918396	1	0.3	0.014	0.06	0.98	2625	-
rs3746631	rs511898	0.337	0.38	0.018	0.04	0.66	5945	-
rs3746631	rs3918392	0.316	1.69	0.088	0.12	0.52	6079	-
rs2787094	rs677044	0.039	0.03	0.001	−0.01	0.25	270	34.15
rs2787094	rs3918400	0.784	3.01	0.14	0.43	0.92	302	-
rs2787094	rs2280090	1	3.02	0.078	0.53	1	1044	-
rs2787094	rs2280091	1	2.57	0.058	0.47	1	1073	-
rs2787094	rs17548872	0.784	3.01	0.14	0.43	0.92	1370	-
rs2787094	rs574174	0.765	10.76	0.421	0.61	0.87	1533	-
rs2787094	rs2853209	0.393	0.7	0.037	0.07	0.66	2311	-
rs2787094	rs528557	0.433	2.39	0.12	0.21	0.6	2581	-
rs2787094	rs3918396	0.762	5.69	0.23	0.52	0.89	2604	-
rs2787094	rs511898	0.283	0.95	0.049	0.07	0.48	5924	-
rs2787094	rs3918392	0.343	0.54	0.027	0.05	0.64	6058	-
rs677044	rs3918400	0.448	1.3	0.062	0.14	0.69	32	52.37
rs677044	rs2280090	1	0.77	0.057	0.12	0.99	774	-
rs677044	rs2280091	0.192	0.02	0.002	0.03	0.94	803	-
rs677044	rs17548872	0.448	1.3	0.062	0.14	0.69	1100	-
rs677044	rs574174	0.002	0	0	0	0.53	1263	-

Contd...

L1	L2	D'	LOD	r^2	CI-low	CI-hi	Dist	T-int
rs677044	rs2853209	0.266	0.81	0.031	0.05	0.48	2041	-
rs677044	rs528557	0.094	0.04	0.001	0	0.46	2311	-
rs677044	rs3918396	0.677	0.66	0.019	0.11	0.91	2334	-
rs677044	rs511898	0.176	0.15	0.005	0.01	0.5	5654	-
rs677044	rs3918392	1	8.58	0.307	0.78	1	5788	-
rs3918400	rs2280090	1	0.85	0.018	0.13	0.99	742	75.37
rs3918400	rs2280091	1	0.51	0.013	0.08	0.98	771	-
rs3918400	rs17548872	1	20.74	1	0.9	1	1068	-
rs3918400	rs574174	1	8.18	0.316	0.77	1	1231	-
rs3918400	rs2853209	1	2.33	0.054	0.43	1	2009	-
rs3918400	rs528557	1	3.97	0.145	0.63	1	2279	-
rs3918400	rs3918396	1	0.47	0.013	0.08	0.98	2302	-
rs3918400	rs511898	0.849	2.31	0.101	0.4	0.96	5622	-
rs3918400	rs3918392	0.535	4.79	0.287	0.33	0.7	5756	-
rs2280090	rs2280091	1	20.48	0.75	0.89	1	29	105.54
rs2280090	rs17548872	1	0.85	0.018	0.13	0.99	326	-
rs2280090	rs574174	1	1.27	0.056	0.21	0.99	489	-
rs2280090	rs2853209	0.451	1.61	0.066	0.17	0.66	1267	-
rs2280090	rs528557	0.891	8.2	0.279	0.69	0.97	1537	-
rs2280090	rs3918396	0.564	0.13	0.01	0.05	0.96	1560	-
rs2280090	rs511898	0.777	5.85	0.204	0.54	0.9	4880	-
rs2280090	rs3918392	0.938	0.25	0.016	0.06	0.97	5014	-
rs2280091	rs17548872	1	0.51	0.013	0.08	0.98	297	83.22
rs2280091	rs574174	1	1.16	0.042	0.19	0.99	460	-
rs2280091	rs2853209	0.573	1.71	0.08	0.22	0.78	1238	-
rs2280091	rs528557	0.926	6.35	0.226	0.68	0.98	1508	-
rs2280091	rs3918396	1	0.38	0.023	0.07	0.98	1531	-
rs2280091	rs511898	0.774	4.1	0.152	0.48	0.91	4851	-
rs2280091	rs3918392	0.334	0.03	0.001	0.03	0.95	4985	-
rs17548872	rs574174	1	8.18	0.316	0.77	1	163	66.94
rs17548872	rs2853209	1	2.33	0.054	0.43	1	941	-
rs17548872	rs528557	1	3.97	0.145	0.63	1	1211	-
rs17548872	rs3918396	1	0.47	0.013	0.08	0.98	1234	-
rs17548872	rs511898	0.849	2.31	0.101	0.4	0.96	4554	-
rs17548872	rs3918392	0.535	4.79	0.287	0.33	0.7	4688	-
rs574174	rs2853209	0.803	3.06	0.111	0.45	0.93	778	99.98
rs574174	rs528557	0.958	12.96	0.422	0.81	0.99	1048	-
rs574174	rs3918396	1	15.58	0.55	0.87	1	1071	-
rs574174	rs511898	0.913	10.64	0.368	0.74	0.97	4391	-
rs574174	rs3918392	0.455	1.39	0.065	0.15	0.7	4525	-
rs2853209	rs528557	0.014	0	0	−0.01	0.2	270	80.79
rs2853209	rs3918396	0.59	0.76	0.033	0.11	0.85	293	-

Contd...

L1	L2	D'	LOD	r^2	CI-low	CI-hi	Dist	T-int
rs2853209	rs511898	0.004	0	0	−0.01	0.18	3613	-
rs2853209	rs3918392	1	2.63	0.054	0.47	1	3747	-
rs528557	rs3918396	1	8.27	0.253	0.8	1	23	80.25
rs528557	rs511898	0.896	28.4	0.771	0.81	0.95	3343	-
rs528557	rs3918392	0.357	0.5	0.018	0.05	0.67	3477	-
rs3918396	rs511898	0.922	6.07	0.207	0.67	0.98	3320	56.9
rs3918396	rs3918392	0.865	0.17	0.01	0.05	0.97	3454	-
rs511898	rs3918392	0.338	0.43	0.016	0.04	0.66	134	5.12

D', D prime between two loci; LOD, log of likelihood odds ratio; r^2, correlation coefficient between two loci; CI-low and CI-hi, 95% confidence interval lower and upper bound on D; Dist, Distance between two loci; T-int, statistic used by HapMap project in Haploview software to measure the completeness of information represented by a set of markers in a region are represented

Table V. Details of significantly associated haplotype single nucleotide polymorphisms (SNPs) of *ADAM33* among controls and asthma patients

SNPs	Block	χ^2	Case-control frequency	Haplotype (*P*)
rs2280090 rs2280091	GA	2.256	0.791, 0.868	0.829
	AG	0.794	0.155, 0.113	0.134
	AA	1.927	0.055, 0.019	0.037
	D' - 1, LOD - 20.48, r^2-0.75, Distance - 29 bp, T-int - 105.54			

D', D prime between two loci; LOD, log of likelihood odds ratio; r^2, correlation coefficient between two loci

A similar study on Korean asthmatics confirmed that both SNPs and SNP haplotypes were associated with bronchial hyper-responsiveness and asthma[33]. No haplotype in linkage disequilibrium was consistent in all populations studied. This could be due to differences in each population's linkage disequilibrium[34]. The minor alleles observed in the two SNPs in the present study (rs2280090 and rs2280091) were in the linkage group with a strong association of 20.48 LOD score. These two SNPs formed the haplotype block and strong LOD value supported the association of this haplotype with asthma.

The two polymorphisms associated with asthma in our study were located on the exonic region of the *ADAM33*. The rs2280090 is a variation wherein the amino acid proline changes to serine and the other SNP rs2280091 naturally codes for amino acid methionine changing to threonine. Both the SNPs are located on the cytoplasmic domain of the gene, and these variations have a potential to change the intracellular signal of the protein that contributes to increasing fibroblast and proliferation of smooth muscles. These two features are characteristic of airway remodelling in asthma[29,34].

These variations were found to be benign in their effect individually, with respect to the predictions of PolyPhen-2 predictor tool. The effect of phosphorylation on the amino acids serine and threonine is high, and these amino acid residues mediate numerous signal transduction pathways[35,36]. Since these variations were found to be together in the participants of the study, these might have combined effect on the protein function.

The study was performed as a pilot study with a limited sample number, which remained the main limitation of this study. The inclusion of 35 SNPs allowed us to perform the haplotype analysis. An association of the two SNPs on haplotype analysis was observed linked with each other with strong LOD score, and since both the SNPs are located on the same domain (cytoplasmic domain) of the ADAM33 protein, it is possible that it may hamper the protein function. This suggests that further functional analysis of this domain may help us to better understand the role of *ADAM33* in asthma.

In conclusion, an association of two polymorphisms (rs2280090 and rs2280091) was found with asthma on haplotype analysis but not to other asthma phenotypes, such as asthma severity and atopy. Since this was a pilot study, the sample size used for the study was small and further studies with larger sample size would be necessary.

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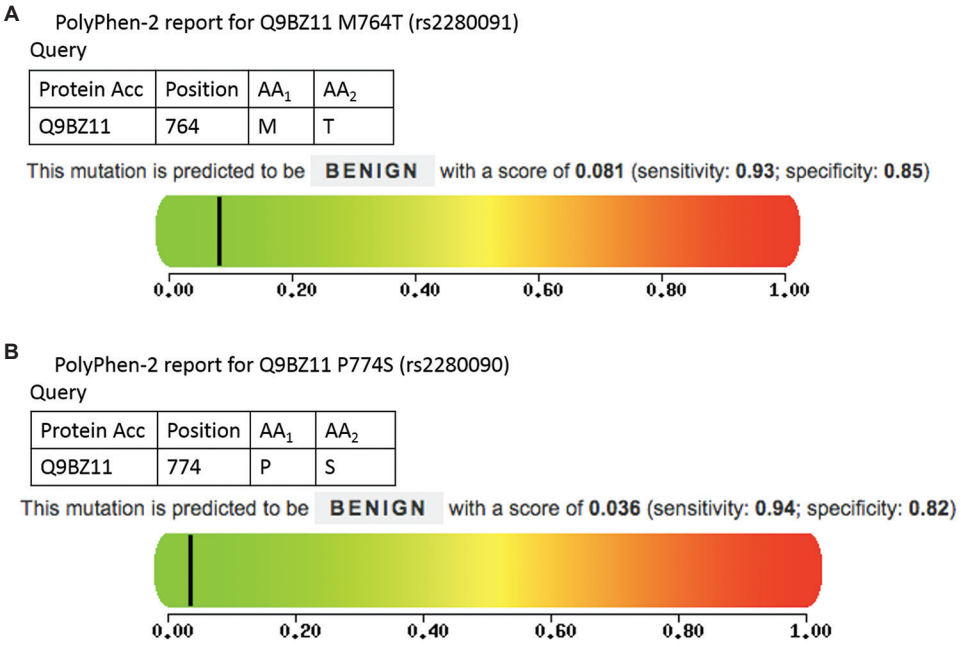

Fig. 2. PolyPhen-2 analysis showing the "Benign" effect prediction individually with (**A**) a score of 0.081 for the SNP rs2280091 (sensitivity: 0.93 and specificity: 0.85) and, (**B**) a score of 0.036 for the SNP rs2280090 (sensitivity: 0.94 and specificity: 0.82).

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***Conflicts of Interest*:** None.

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