

Analysis of ADRB2 (Arg16Gly) Gene Variant with Susceptibility, Pharmacogenetic Response and Disease Severity in South Indian Asthmatics

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Abstract— β 2-Adrenergic receptor (β 2-AR) plays a crucial role in asthma pathophysiology by regulating, processes of the lung function, and clinical response to bronchodilators. The +46G>A- Gly16Arg polymorphism in the gene encoding β 2 adrenergic receptor (ADRB2) has been associated with receptor non-responsiveness after β 2-agonist exposure. In the present study, we sought to evaluate the possible association of Gly16Arg polymorphism with asthma susceptibility, pharmacogenetic response to Salbutamol, and varying degrees of disease severity. Three hundred ninety-eight clinically diagnosed patients and 456 healthy controls were enrolled for the study. Patients were classified into severity classes according to Global Initiative for Asthma guidelines. To assess bronchodilator response, spirometry was performed before and 15 min after Salbutamol (200 μ g) delivery. Responders to Salbutamol were categorized if percentage reversibility was greater than or equal to 12 % in them, while those showing reversibility less than 12 % were classified as non-responders. Genotyping was carried out by ARMS-PCR technique. Statistical methods were applied to test for the significance of the results. In the present study, there was lack of significant association of polymorphism with disease susceptibility as well as with bronchodilator response. The polymorphism was not associated with mild and moderate asthma subtypes; however, there was a notable association with severe asthma subtype. In addition, the polymorphism was associated with severe asthma compared to subtypes of mild and moderate asthma combined. In a South Indian population, the ADRB2 Arg/Gly may not form a susceptible variant to develop asthma nor can be a standard predictive marker to bronchodilator response; nevertheless, the patterns in asthma severity can be predicted by analyzing this variant.

KEY WORDS: bronchial asthma; Arg16Gly variant; asthma susceptibility; Salbutamol response; asthma severity.

INTRODUCTION

Asthma is a complex inflammatory disorder that currently affects about 300 million people worldwide, and

approximately 250,000 people die of asthma annually [1]. According to the cross-sectional National Family Health Survey (NFHS)-3 (IIPS and Macro International, 2007), 25–30 million people have been positively diagnosed for asthma in India and around two out of every 100 individuals have asthma in South Indian states [2].

There has been substantial increase in the treatment options in the management of asthma, of which β 2-agonists hold prime importance. Inhaled β 2-agonists have been established as first-line treatment for the management of acute asthma because of their excellent bronchodilator effects, wide therapeutic range, and low level of side effects [3, 4]. β 2-Agonists in combination with inhaled corticosteroids form an effective and endures well-tolerated treatment regimen in patients with persistent

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asthma [5–7] which is regularly recommended by asthma treatment guidelines [8–11].

A considerable fraction of the interindividual variability in response to short-acting β_2 -agonist treatment is attributed to gene polymorphism especially in the ADRB2 gene [12–17]. ADRB2 gene is an intron-less gene located on chromosome 5q3116 that codes for β_2 -adrenergic receptor (β_2 -AR)—a GPCR protein. β_2 -Agonist-induced activation of the β_2 -AR causes the dilatation of airway smooth muscle, thereby providing desired therapeutic effect [18]. A number of studies have examined the pharmacogenomics and pharmacogenetics of β_2 -agonists associating ADRB2 polymorphism. In studies utilizing site-directed mutagenesis and recombinant expression, three loci at amino acid positions 16 (Gly16Arg), 27 (Gln27Glu), and 164 (Thr164Ile) have been found to have a significant impact on the functioning of the receptor [19, 20]. Of particular prominence, the coding SNP at the 46th (A→G, rs1042716) position of the ADRB2 gene that substitutes Arg to Gly at the position 16 in the β_2 -AR has been associated with receptor non-responsiveness after β_2 -agonist exposure [19]. Recent literature reviews suggest an emerging consensus that the Arg16 allele is associated with clinically significant and unsatisfactory response to beta-agonists [21, 22]. Results from the Beta Agonist Genotype Study (BAGS) showed that β_2 16-Arg/Arg homozygous subjects had a small decrease in morning peak expiratory flow (PEF) when receiving regular Salbutamol, but no change was observed with Salbutamol used on an as-needed basis [23]. However, in some ethnic population, there has been conflicting reports showing no change in bronchodilator response to short-acting β_2 -agonist [24, 25] in asthmatics with varying β_2 -AR genotypes.

Emerging evidence from different ethnic groups from the world has attributed Arg/Gly variant with variable response and severity; however, the reports from Indian subcontinent remain undocumented; hence, the present study centers to identify the possible association of Gly16Arg polymorphism with asthma susceptibility, response to β_2 -agonist (Salbutamol) therapy and severity.

MATERIALS AND METHODS

Study Population

Three hundred ninety-eight patients diagnosed by physician were recruited from Government General and Chest Hospital (Gandhi Medical College and Osmania Medical College, Hyderabad, India). No patient had

evidence of chronic obstructive pulmonary disease, bronchiectasis, or any other lung disorder. Only adults 20 years and above were recruited in the study. Since children do not cooperate well with pulmonary function test, which may lead to false-positive results in lung function variables, they were excluded from the present study. Epidemiological details such as age, gender, age of onset, addictions, inhabitation, and allergic information were taken through a personal interview with the help of a standard questionnaire. A total of 456 age-, gender-, and ethnicity-matched participants who neither had evidence nor family history of asthma served as healthy controls. Patients and controls were recruited from same demographic area to rule out any discrepancies in ethnicity. The study protocol was approved by the ethical committee at our institution. An informed and voluntary consent was obtained from all the subjects selected for the study.

Assessment of Bronchodilator Response and Severity

To assess the bronchodilator response, inhalation of the 200 μ g of Salbutamol (Asthalin, Cipla ©) was administered using a metered dose inhaler and a spacer. Spirometry (Viasys Healthcare, Micromedical Limited, Kent, England) was performed before and 15 min after Salbutamol dose, according to the American Thoracic Society Standards [26]. Pulmonary function test results were expressed as a percentage of the predicted normal value using Hankinson's age-adjusted and race-adjusted prediction equation [27]. The percentage reversibility change in the forced expiratory volume (FEV_1) was calculated by Knudson equation [28] as given below:

%Reversibility

$$= \frac{\text{Max. } FEV_1 \text{ after drug} - \text{Max. } FEV_1 \text{ before drug}}{\text{Max. } FEV_1 \text{ before drug}} \times 100$$

Responders to Salbutamol were those patients who demonstrated percentage reversibility greater than or equal to 12 %, while patients demonstrating reversibility less than 12 % were categorized as non-responders [29]. Patients were classified into three severity groups based on FEV_1 % predicted as having mild ($FEV_1 > 80$ %), moderate (FEV_1 60 to 80 %), or severe ($FEV_1 < 60$ %) as defined by Global Initiative for Asthma guidelines (GINA, 2014, National Heart, Lung, and Blood Institute, National Institutes of Health, USA, and the World Health Organization.).

Genotyping of ADRB2 ARG/Gly Polymorphism

Genomic DNA was isolated from whole blood samples of all the patients and control subjects by the salting-out procedure [30]. The genotype for Arg/Gly (nucleic acid position 46, amino acid position 16, rs1042713) was determined by means of allele-specific polymerase chain reaction method. Briefly, two complementary reactions were established for each allele consisting of target DNA and allele-specific ARMS primers. A 913-bp region in exon 1 of the ADRB2 gene that spans the polymorphism was targeted for amplification. The primers used were as follows: antisense (common forward) 5'-CCA ATT TAG GAG GAT GTA AAC TTC-3', sense (reverse "A" allele specific) 5'-CTT CTT GCT GGC ACC CAA TA-3', and sense (reverse "G" allele specific) 5'-CTT CTT GCT GGC ACC CAATG-3'. The optimized reaction mixture of 20 μ l contained 100 ng of genomic DNA, 1 $\times$ reaction buffer, 1.5 μ M MgCl₂, 30 μ M of each dNTP, 0.16 μ M of each primer, and 0.3 U of Taq DNA polymerase. The PCR amplification reaction consisted of initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 64 °C for 20 s, and 72 °C for 30 s. The final extension step was at 72 °C for 6 min. The PCR products were electrophoresed on 2 % agarose gel stained with ethidium bromide and were visualized under ultraviolet light with a 100-bp ladder (Fig. 1).

Statistical Analysis

Hardy–Weinberg equilibrium was tested for ADRB2 Arg/Gly variant. Any deviation in the allele and genotype

frequencies between patients and healthy controls, response phenotypes, and within severity groups were tested for statistical significance using Yates corrected χ^2 test and odds ratio at 95 % C.I. Lung function end points were compared using ANOVA and Student test (with Hartley's f test for equality of variance). All the p values were two sided, and the level of significance was considered at $p < 0.05$. Statistical analyses were performed using Open EPI online statistical software [31] (Open EPI v 2.3.1, from department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA 30322, USA) and SNP stat web server (http://bioinfo.iconcologia.net/en/SNPStats_web) [32].

RESULTS

A total of 398 physician diagnosed asthmatics and 456 healthy controls were recruited for the study. The demographic characteristics of patients and controls are presented in Table 1. In the study, there was no significant difference in number of males and females in patient and control groups; however, the number of females was marginally higher in both the groups. Non-smokers were significantly higher in both cases and controls (p value = 0.0060). The mean duration from which the patients were suffering was found to be 10.5 years. One hundred sixty-five (41.45 %) patients had a family history of asthma whereas 233 (58.55 %) showed absence of family history. Out of 398 asthma subjects, 102 (25.63 %), 127 (31.91 %), and 169 (42.46 %), respectively, belonged to mild,

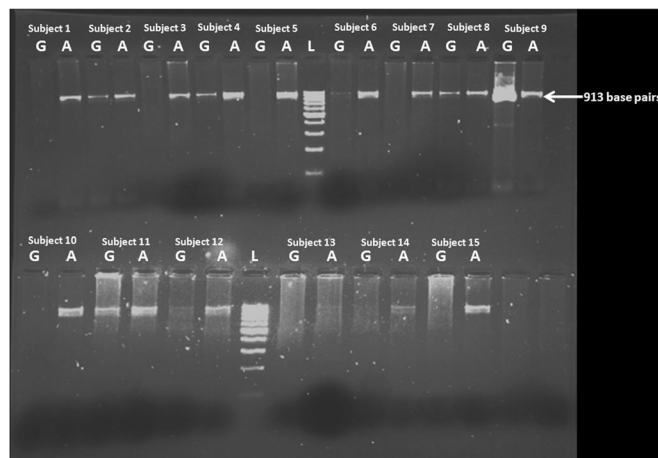


Fig. 1. A 913-bp region in exon 1 of the ADRB2 gene amplified by allele-specific PCR. Two adjacent lanes are specified for genotyping for each subject (labeled as *subject 1* and so on). Amplified bands in the lane marked with only *G* among the two lanes specified for subject represents GG (Arg/Arg) genotype of the subject. Likewise, amplified bands in the lane marked with only *A* among the two lanes specified for subject represents AA (Gly/Gly) genotype of subject. While bands in the both *G* and *A* lanes represent subject is heterozygous with GA (Arg/Gly) genotype. *L* is 100-bp ladder.

Table 1. Demographic Variables of Asthmatics and Controls

	Patients <i>N</i> =398	Controls <i>N</i> =456	χ^2	OR (95 % CI)	<i>p</i> value
Gender	<i>n</i> (%)	<i>n</i> (%)			
Males	185 (46.48)	187 (41.00)	ref		
Females	213 (53.52)	269 (59.00)	2.372	1.249 (0.952–1.639)	0.123
Age (in years) ^a					
Males	46.44±11.24	47.04±10.64	ref		
Females	42.24±09.85	44.84±08.46	0.00007136	1.048 (0.584–1.879)	0.993
Smoking status	<i>n</i> (%)	<i>n</i> (%)			
Smokers	132 (33.17)	194 (42.54)	ref		
Non-smokers	266 (66.83)	262 (57.46)	7.527	0.670 (0.506–0.886)	0.006
Duration of asthma (in years) ^a	10.5±3.00	–	–	–	–
Family history of bronchial asthma	<i>n</i> (%)				
Present	165 (41.45)	–	–	–	–
Absent	233 (58.55)	–	–	–	–

^a Figures expressed as mean

moderate, and severe classes of asthma. Further, among 398 asthmatics, 261 (65.58 %) patients were responders to Salbutamol while 137 (34.42 %) patients were non-responders. The lung function variables (FEV₁, PEF, and FVC) with respect to severity classes and response phenotype are represented in Table 2. We found significant difference in the values of lung function end points especially in FEV₁ within the severity subsets (ANOVA for FEV₁ within severity subtype; *F* Stat=6.682, *p* value=0.016, *F* Crit=4.300) and response phenotypes (*t* test for FEV₁ within response phenotype; *F* Stat=2.54, *p* value<0.001). In addition, the mean FEV₁ percentage reversibility was significantly declined in non-responding patients confirming these patients demonstrating refractoriness to Salbutamol (*t* test for mean percentage FEV₁ reversibility within response phenotype; *F* Stat=2.91, *p* value<0.001).

Arg/Gly genotypic and allelic distribution between patients and healthy controls and between responders and

non-responders is shown in Table 3. In the present study, we found no association of the polymorphism in asthmatics relative to healthy controls. Furthermore, none of the inheritance model demonstrated significant association of the Arg/Gly polymorphism to asthma susceptibility. (controls vs. patients, Arg/Arg vs. Gly/Gly, OR=0.881 at 95 % C.I. (0.631–1.230), *p*=0.509, χ^2 =0.434; Arg vs. Gly, OR at 95 % C.I.=0.917 (0.758–1.110), *p*=0.4025, χ^2 =0.703).

At the similar note, none of the inheritance models supported the association of the polymorphism with response phenotype. (responders vs. non-responders, Arg/Arg vs. Gly/Gly, OR=0.763 at 95 % C.I.=(0.463–1.257), *p*=0.3490, χ^2 =0.877; Arg vs. Gly, OR=0.821 at 95 % C.I.=(0.621–1.102), *p*=0.215, χ^2 =1.538). Therefore, the study implies the polymorphism may not contribute to asthma pathogenesis nor can be a confounding factor to determine pharmacogenetic response to Salbutamol at least in a South Indian population.

Table 2. Lung Function Variables in Asthmatics With Varying Degrees of Severity (Gina Classification) and Response Phenotypes

	<i>n</i> (%)	FEV ₁ (mean±SD)	PEF (mean±SD)	FVC (mean±SD)
Severity subtype				
Mild	102 (25.63)	73.42±3.62	57.76±4.54	68.24±1.24
Moderate	127 (31.91)	65.12±4.64	52.45±4.23	64.77±6.91
Severe	169 (42.46)	44.83±4.21	36.55±6.13	52.27±3.73
Response phenotype				
Responders	261 (65.58)	64.82±5.42	48.84±4.85	51.26±2.40
Non-responders	137 (34.42)	52.66±3.4	40.21±6.21	51.55±4.21
Mean percentage FEV ₁ reversibility (mean%±SD)				
Responders	25.55±4.1			
Non-responders	10.15±2.4			

ANOVA for FEV₁ within severity subtype *F* Stat=6.682; *p* value=0.016; *F* Crit=4.300; *t* test for FEV₁ within response phenotype *F* Stat=2.540; *p* value<0.001; *t* test for mean percentage FEV₁ reversibility within response phenotype *F* Stat=2.910; *p* value<0.001

Table 3. Genotype and Allelic Distribution in Asthmatics and Controls and Responders and Non-responders

Inheritance model	Genotype	Control vs. patients		Patients: responders vs. non-responders							
		Control N=456 n %	Patients N=398 n %	OR (95 % C.I.)	χ ²	p value	Responders N=261 n %	Non-responders N=137 n %	OR at (95 % C.I.)	χ ²	p value
Co-dominant	Arg/Arg	143 (31.4)	144 (36.2)	ref			87 (33.3)	57 (41.6)	ref		
	Arg/Gly	171 (37.5)	128 (32.2)	0.743 (0.537–1.029)	2.904	0.080	90 (34.5)	38 (27.7)	0.644 (0.388–1.068)	2.501	0.113
	Gly/Gly	142 (31.1)	126 (31.7)	0.881 (0.631–1.230)	0.434	0.509	84 (32.2)	42 (30.7)	0.763 (0.463–1.257)	0.877	0.349
Dominant	Arg/Arg	143 (31.4)	144 (36.2)	ref			87 (33.3)	57 (41.6)	ref		
	Arg/Gly- Gly/Gly	313 (68.6)	254 (63.8)	0.806 (0.606–1.071)	2.003	0.157	174 (66.7)	80 (58.4)	0.709 (0.458–1.075)	2.316	0.128
Recessive	Arg/Arg- Arg/Gly	314 (68.9)	272 (68.3)	ref			177 (67.8)	95 (69.3)	ref		
	Gly/Gly	142 (31.1)	126 (31.7)	1.024 (0.766–1.368)	0.007	0.920	84 (32.2)	42 (30.7)	0.932 (0.596–1.456)	0.039	0.843
Allele frequencies	Arg	457 (50.0)	416 (52.0)	ref			264 (51.0)	152 (55.0)	ref		
	Gly	455 (50.0)	380 (48.0)	0.917 (0.758–1.110)	0.703	0.402	258 (49.0)	122 (45.0)	0.821 (0.612–1.102)	1.538	0.215

C.I. confidence interval, OR odds ratio

Table 4 shows the genotypic and allelic distribution within the severity groups. In the study, it was observed that there was an increasing trend of recessive genotype as well as recessive allele coherent with increasing degrees of severity. The frequencies of recessive genotype Gly/Gly in mild, moderate, and severe classes were 24.5, 28.4, and 38.5 %, respectively. Similarly, the recessive allele followed a percentage increasing trend of 42, 44, and 54 %, respectively, corresponding to mild, moderate, and severe classes. When mild and moderate subtypes were compared for the genotypic and allele frequencies, we found no significant difference in the distribution. (mild vs. moderate, Arg/Arg vs. Gly/Gly, OR=1.186 at 95 % C.I.=(0.616–2.280), $p=0.7299$, $\chi^2=0.1192$; Arg vs. Gly, OR=1.104 at 95 % C.I.=(0.760–1.603), $p=0.669$, $\chi^2=1.182$). However, when moderate and severe classes were compared for the presence of recessive genotype and allele, we found there was significant association of the recessive allele in severe asthma than in moderate subtypes. (moderate vs. severe, Arg/Arg vs. Gly/Gly, OR=1.806 at 95 % C.I.=(1.029–3.169), $p=0.054$, $\chi^2=3.705$; Arg vs. Gly, OR=1.497 at 95 % C.I.=(1.079–2.076), $p=0.019$, $\chi^2=5.461$). In addition, the mutation was significantly associated with severity subgroups than their milder counterparts. (mild vs. severe, Arg/Arg vs. Gly/Gly, OR=2.141 at 95 % C.I.=(1.156–3.965), $p=0.022$, $\chi^2=5.230$; Arg vs. Gly, OR=1.653 at 95 % C.I.=(1.164–2.348), $p=0.006$, $\chi^2=7.43$).

In the further analysis, when the frequencies of genotype and allele were compared between severity subset to their mild and moderate counterparts combined, we observed a statistical significance confirming that recessive allele as well as the recessive genotype was associated with severe asthma (mild+moderate vs. severe Arg/Arg vs. Gly/Gly, OR=1.943 at 95 % C.I.=(1.192–3.167), $p=0.010$, $\chi^2=6.526$; Arg vs. Gly, OR=1.564 at 95 % C.I.=(1.179–2.075), $p=0.002$, $\chi^2=9.213$).

In order to find whether normal allele/genotype was showing any bias towards milder phenotype or rather mutant allele was showing any bias towards severity phenotype, we performed a chi-squared analysis. We found that though the genotypes did not show much bias towards any of the severity subtype (mild vs. moderate vs. severe $p=0.1033$, $\chi^2=7.697$, $df=4$), but the wild allele “Arg” was fairly biased towards milder phenotype or rather recessive allele “Gly” was fairly biased towards severity subtype (mild vs. moderate vs. severe $p=0.0007$, $\chi^2=9.922$, $df=2$). From the study, we may perhaps speculate that the higher frequency of recessive allele may lead to asthma severity.

Table 4. Genotypic and Allelic Distribution Within Glna Classified Severity Subsets

Inheritance model	Genotype	Mild N=102 n %	Moderate N=127 n %	Severe N=169 n %	Mild vs. moderate	Mild vs. severe	Moderate vs. severe	Severe vs. mild and moderate
Co-dominant	Arg/Arg	42 (41.2)	51 (40.2)	51 (30.2)	ref	ref	ref	ref
	Arg/Gly	35 (34.3)	40 (31.5)	53 (31.4)	OR=0.941 C.I. =0.511-1.733 χ^2 =0.0015 $p=0.968$	OR=1.247 C.I. =0.690-2.251 χ^2 =0.339 $p=0.560$	OR=1.325 C.I. =0.753-2.331 χ^2 =0.694 $p=0.404$	OR=1.289 C.I. =0.789-2.104 χ^2 =0.791 $p=0.373$
Dominant	Gly/Gly	25 (24.5)	36 (28.4)	65 (38.5)	OR=1.186 C.I. =0.616-2.280 χ^2 =0.119 $p=0.729$	OR=2.141 C.I. =1.156-3.965 χ^2 =5.230 $p=0.022^*$	OR=1.806 C.I. =1.029-3.169 χ^2 =3.705 $p=0.054$	OR=1.943 C.I. =1.192-3.167 χ^2 =6.526 $p=0.010^*$
Recessive	Arg/Arg	42 (41.2)	51 (40.2)	51 (30.2)	ref	ref	ref	ref
	Arg/Gly- Gly/Gly	60 (58.8)	76 (59.8)	118 (69.8)	OR=1.043 C.I. =0.613-1.773 χ^2 =0.0004 $p=0.983$	OR=1.620 C.I. =0.969-2.706 χ^2 =2.943 $p=0.086$	OR=1.553 C.I. =0.957-2.518 χ^2 =2.771 $p=0.096$	OR=1.58 C.I. =1.038-2.411 χ^2 =4.144 $p=0.041^*$
Allele frequencies	Arg	77 (75.5)	91 (71.7)	104 (61.5)	ref	ref	ref	ref
	Gly/Gly	25 (24.5)	36 (28.4)	65 (38.5)	OR=1.218 C.I. =0.673-2.206 χ^2 =0.252 $p=0.615$	OR=1.925 C.I. =1.114-3.328 χ^2 =4.971 $p=0.0258^*$	OR=1.580 C.I. =0.963-2.592 χ^2 =2.866 $p=0.090$	OR=1.721 C.I. =1.124-2.637 χ^2 =5.749 $p=0.016^*$
Severe vs. mild vs. moderate	Arg	119 (58.0)	142 (56.0)	155 (46.0)	ref	ref	ref	ref
	Gly	85 (42.0)	112 (44.0)	183 (54.0)	OR=1.104 C.I. =0.760-1.603 χ^2 =0.182 $p=0.669$	OR=1.653 C.I. =1.164-2.348 χ^2 =7.43 $p=0.006^*$	OR=1.497 C.I. =1.079-2.076 χ^2 =5.461 $p=0.019^*$	OR=1.564 C.I. =1.179-2.075 χ^2 =9.213 $p=0.002^*$
Genotypes	χ^2	p value	df					
Alleles	7.697	0.1033	4					
	9.922	0.0070	2					

C.I. confidence interval, OR odds ratio, df degrees of freedom

* $p<0.05$

DISCUSSION

Recent understanding of genetics has now recognized that asthma does not follow simple Mendelian mode of inheritance and is now surfaced as a multifactorial disorder of the airways brought about by complex interaction between genetic and environmental factors [33].

Regulation of ADRB2 contributes to overall pathological condition in asthmatic lung; however, as evident from previous studies, it is worthy to note that defects in the ADRB2 gene do not form a confounding factor to develop asthma condition and the present study is in coherence to this observation. Our study demonstrating lack of association of ADRB2 polymorphism with asthma susceptibility is supported by *in vivo* studies of the genetic variants in ADRB2 which suggests that Arg/Gly polymorphisms are not likely to be disease-causing but possibly serve as predictive markers for responsiveness to beta2-agonists [34]. Furthermore, reports from three meta-analyses of common non-synonymous SNPs in ADRB2 have concluded that the polymorphisms are not associated with the diagnosis of asthma in different ethnic groups [35, 36].

ADRB2 codes for B2-AR which is a transmembrane protein are only activated on exposure to agonist and do not participate in the bronchial hyperresponsiveness observed in the inflammatory process occurring in asthma. Since bronchial asthma is an inflammatory disorder, this perhaps may explain why ADRB2 polymorphism forms a considerable indicator for drug responsiveness but not a susceptibility gene in the development of asthmatic conditions.

In the further perusal, we pursued to analyze the possible association of Arg16Gly polymorphism with Salbutamol (200 µg) responsiveness. In the study, there was no notable association of the polymorphism with the drug response. There always have been contradicting results emerging from different ethnic populations associating the drug response polymorphism. For example, our study is supported by investigation in New Zealanders and in White and African American population, wherein there was no association of Arg/Gly SNP with variable drug response [20, 36–38].

Study conducted by Bleecker and colleagues in 2006 found that there was no indication that the Arg/Arg genotype was responsible for a therapeutic outcome different from that of any other genotype, neither for asthma exacerbations nor for day-to-day asthma control measures such as lung function, as-needed symptom relief, or asthma symptoms [39]. In addition, study conducted by Martin *et al.* in 2008, on Australian inhabitants showed that the

polymorphism was associated only with significant response to higher dose of short-acting beta2-agonists, while lower dose of drug did not affect the response outcome [40]. In contrast to our study, several investigations have revealed that homozygotes of Arg16 are more likely to respond (more rapid response and increased forced expiratory volume in 1 s) to short-acting beta2-agonists (SABA) compared to homozygote of Gly16 and heterozygotes [41–43].

Since, bronchial asthma is a heterogeneous disorder, even in patients with an apparently identical clinical phenotype, response to drug treatment may be remarkably variable. It is common for some patients to respond in a salutary fashion to a given treatment, while others fail to manifest such a response. The basis for variable treatment response in different ethnic populations still remains elusive; however, it may be interpreted that correlations between ADRB2 variants and beta2-agonists may be specific to particular type of beta2-agonist. For example, some studies have used short-acting beta2-agonists (SABAs) while others have used long-acting beta2-agonists (LABAs) to predict the bronchodilator response [23, 44–46]. In addition, pharmacogenetic correlations may also be affected by the interval of drug treatment (regular use or use as needed) and interactions with other medications. Furthermore, these correlations may also be specific to certain ethnicities and subject to sex effects [47].

In the study, we observed the increasing frequency of recessive allele was coherent with increasing degree of severity. Although, the recessive genotypes and recessive allele were not significantly associated with mild subtypes when compared with moderate subtypes; nevertheless, there was a notable association of the recessive genotype and recessive allele which was prominent in severe subtypes when compared with mild or moderate subtypes. In addition, there was significant association of recessive genotype and allele with severe asthma in comparison with mild and moderate asthma combined. In the further approach, we performed chi-squared analysis to find whether recessive allele or genotype had a bias for severe subtypes. Although the recessive genotypes did not show much bias towards any of the severity subtype, however, recessive allele was appreciably biased towards severe asthma than in mild or moderate subtype.

In the study, we observed that the recessive allele offers an elevated risk to develop asthma severity. We found that the recessive allele offers 1.21-fold greater risk in severe subsets than in their mild and moderate counterparts combined: thereby, it may be said that it promotes severe disease process in asthma. Therefore, from the

extensive analysis as above, we concluded that the recessive genotypes and allele may increase the severity of asthma disease process in the South Indian cohorts. In coherence to our study, Turki *et al.* also attributed Arg/Gly polymorphism to increase the severity in American population of Ohio, USA [48]. In addition, in Caucasian population, Weir *et al.* showed that the polymorphism predisposes as a risk factor for fatal/near-fatal or severe asthma [49].

The exact relation between Arg/Gly polymorphism with severity still remains obscure; nevertheless, studies have shown that patients with a recessive allele often represent nocturnal asthma symptoms, higher airway reactivity, and decreased response to β 2-agonist therapy [50], and these representations may perhaps lead to severe asthma conditions. It has also been suggested that individuals who die of asthma represent a group of Gly/Gly homozygotes [51].

There have been inconsistent correlations describing the association of asthma susceptibility, pharmacogenetic response, and severity with Arg/Gly polymorphism in different ethnic groups. A meta-analysis by Contopoulos-Ioannidis *et al.* showed that most associations of the polymorphisms in ADRB2 and asthma drug response and severity phenotypes are statistically insignificant because of small sample sizes and less than 2 % of the associations were replicated by two or more groups [52]. In addition, similar beta2-agonists (with standard dose) were not used to predict the pharmacogenetic response which may further add discrepancy in the reports across studies in different ethnic populations.

We report the first ever study from South Indian population to analyze the effect of Arg/Gly polymorphism on asthma susceptibility, pharmacogenetic response to beta2-agonists, and severity. The study however needs replication in different ethnic populations to arrive at absolute rationales, behind ADRB2-related asthma pathophysiology, thereby ruling out any inconsistencies.

CONCLUSION

In summary, in the context of South Indian population, the ADRB2 Arg46Gly is not a susceptible variant to developing asthma risk. In addition, the polymorphism was not associated with pharmacogenetic outcome; therefore, it may not stand as a predictive marker to bronchodilator response. However, by analyzing this variant, the patterns in asthma severity can possibly be predicted.

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