RESEARCH PAPER

ADRB2 Polymorphism and Salbutamol Responsiveness in Northern Indian Children with Mild to Moderate Exacerbation of Asthma

PUNEET KAUR SAHI, SHIVARAM SHASTRI, RAKESH LODHA, NEERJA GUPTA, #RM PANDEY, SUSHIL KUMAR KABRA AND MADHULIKA KABRA

From Departments of Pediatrics and #Biostatistics, All India Institute of Medical Sciences, New Delhi, India.

Correspondence to: Dr Madhulika Kabra, Professor, Division of Genetics, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India. madhulikakabra@hotmail.com

Objectives: The primary objective was to determine the association between beta-2 *adrenergic receptor* (*ADRB2*) gene polymorphism (rs1042713, c.46A>G, p.Arg16Gly) and the response to inhaled salbutamol in North Indian children aged 5 to 15 years, with mild to moderate exacerbation of asthma.

Methods: This cross-sectional study was done at a tertiary-care hospital in Northern India from June 2011 to May 2013. 120 children with asthma with mild to moderate exacerbation underwent spirometry at baseline and after administration of three doses of salbutamol. An increase in $FEV_1 \geq 15\%$ was considered as positive response. Blood samples from these children were analysed for *ADRB2* polymorphism (p.Arg16Gly). 94 non-asthmatic adult controls were also studied to determine the prevalence of *ADRB2* polymorphism.

Results: In asthmatic children, the frequency of AA, GG, AG genotypes were 24.2%, 24.2% and 51.7% compared to 20.2%, 20.2 % and 59.6%, respectively in the non-asthmatic adults. Salbutamol responsiveness showed no correlation with the studied *ADRB2* polymorphism (p= 0.55). A trend towards greater bronchodilator responsiveness amongst AA genotype, compared to GG genotype was observed (Median change in percent predicted FEV_1 14.5% and 7.5%, respectively).

Conclusions: No correlation was found between salbutamol responsiveness and *ADRB2* genotype in Northern Indian children with asthma with mild-to moderate exacerbation.

Keywords: *Adrenergic receptor, Bronchodilation, Management, Prediction.*

Asthma affects around 6-31% of Indian children [1]. Short-acting beta-2 agonists (SABA) form the mainstay of treatment of acute exacerbations. However, a significant heterogeneity exists in response to inhaled SABA, of which 70-80% may have a genetic basis [2]. SABA act by binding to the beta 2 adrenergic receptor (ADRB2), coded by an intron-less gene on chromosome 5. A single nucleotide polymorphism (SNP) at nucleotide position 46 of this gene (AGA to GGA) substitutes the 16th amino acid of the translated protein chain from arginine to glycine and alters receptor function. This p.Arg16Gly polymorphism has been the subject of intense research. Conflicting data exist regarding effect of this polymorphism on salbutamol response particularly with regard to different ethnicities. There is a paucity of Indian data, especially in children. Thus, our first objective was to determine an association between Salbutamol response and p.Arg16Gly polymorphism of *ADRB2* gene in children with asthma with acute exacerbation. The second was to determine prevalence of these genotypes in the asthmatic and non-asthmatic population.

METHODS

This cross-sectional study was carried out between June 2011 to May 2013 at a tertiary care hospital in India. The protocol was approved by the Institute's ethics committee and written informed consent was taken from the parents of all participating children, and from all adults enrolled as controls. Adults were chosen as controls, considering that children appearing healthy currently might develop asthma symptoms over a period of time.

Calculation of sample size was based on the observation that compared to homozygotes for Gly-16, homozygotes for Arg-16 were 5.3 times more likely to respond to salbutamol [3]. The prevalence of the Arg16 polymorphism in the general population is shown to be 30-50% [4]. With 50% precision, confidence interval of 95%, sample size was calculated to be 120 [5]. For controls, we planned to enroll 120 adults.

Children between 5-15 years with physician- diagnosed asthma (three or more episodes of reversible airway obstruction documented by bronchodilator response) were screened for enrolment. Those with mild to moderate acute exacerbation, as determined by the Clinical Asthma Score (CAS) [6] were included. Children with life threatening asthma, pre-existing chronic medical conditions, use of long-acting beta agonists in prior 2 weeks, salbutamol therapy by any route in 6 hours preceding the evaluation, and those on oral steroid therapy were excluded.

Copyright of Indian Pediatrics 2016
For personal use only. Not for bulk copying or unauthorized posting to listserv/websites

Clinical details and CAS were recorded in a pre-designed structured proforma. All enrolled children underwent spirometry using a portable spirometer (Superspiro MK2 Micro Medical Ltd, UK), as per standard technique. The absolute and percentage predicted values of following parameters were recorded: FEV_1 (Forced Expiratory Volume-1 second); FVC (Forced Vital Capacity) PEFR (Peak Expiratory Flow Rate) FEF_{50} (Forced Expiratory Flow 50). Thereafter, they received salbutamol 100 µg 2 puffs with MDI (metered dose inhaler) with spacer every 10 min for a total of three doses followed immediately by repeat spirometry. The highest FEV which was available from all adequate curves produced during baseline and post treatment spirometry were recorded. Percentage increase in actual FEV was measured using the formula [(FEV (post-bronchodilator) – FEV baseline)/FEV baseline] X 100. Positive bronchodilator response was considered if percentage increase in FEV_1 was ≥15%.

For DNA analysis, 3-5 mL blood was collected in EDTA vacutainers and stored at 4ºC. DNA was extracted using the phenol chloroform method [7]. A 168 bp region flanking the p.Arg16Gly polymorphism region was amplified using Polymerase Chain reaction (PCR). The forward primer: 5' GCC TTC TTG CTG GCA CCC CAT 3' (21 bases) and reverse primer: 5' CAG ACG CTC GAA CTT GGC CAT G 3' (22 bases) were used. PCR reactions were carried out in 25 µL mix containing 2.5 µL of 10X PCR buffer, 2.5 µL of 2 mM dNTPs, 1.0 µL of 10 µM forward and reverse primer each, 0.25 µL of 3 U/µL *Taq polymerase*, 16.75 µL of sterile water and 1 µL of extracted DNA (100 ng/mL). The above mix was kept in a thermal cycler with set temperature conditions repeated for 35 cycles. Initial denaturation was done at 94°C for 2 minutes followed by denaturation at 94°C for 40 seconds, annealing at 64ºC for 40 seconds, extension at 72°C for 50 seconds and final extension at 72ºC for 5 minutes. A blank, with all components except the DNA template, was run simultaneously with each run of PCR as control. The PCR products were checked for adequate DNA amplification by a run at 150 volts in a horizontal gel electrophoresis system. The underlined bases in both the forward and reverse primers were modified from A to C to create restriction sites for the *NcoI* enzyme which was later used in restriction digestion of the PCR products. The forward primer creates an *Nco*I restriction site on Gly16 PCR product but not on Arg16 PCR product. The reverse primer creates a restriction site on both, thus serving as a control to assess whether restriction digestion was complete.

The amplified PCR products were subjected to restriction digestion with *NcoI* enzyme. 8.8 µL of PCR amplified DNA, 1.0 µl of 1X NE buffer 3, 0.25 µL of *NcoI* enzyme (10,000 U/mL) were mixed and incubated at 37ºC for 16 hours. The products of restriction digestion along with a control (unrestricted PCR product) were run at 150 volts in a horizontal electrophoresis system on a 4% agarose gel. DNA ladder was run to judge the size of the cut products. The gel was observed under UV light and the image was stored.

After restriction digestion of the PCR products of 168 bp: Homozygote AA give 146 bp and 22 bp products; Heterozygotes AG give 146 bp, 128 bp, 22 bp and 18 bp products; Homozygotes GG give 128 bp, 22 bp and 18 bp products.

Data were collected in a structured proforma and managed using MS Excel software. Statistical analysis was done using STATA 11. The comparison of salbutamol responsiveness of children with different *ADRB2* genotypes (AA/AG/GG) was done using the Pearson Chi square test. P value less than 0.05 was considered statistically significant. Hardy-Weinberg equilibrium analysis of *ADRB2* SNP at 46th nucleotide position was also performed [8].

RESULTS

During the study period, 246 children were screened for inclusion in to the study. Of these, 120 results were finally available for analysis (***Fig. 1***). The baseline characteristics of the study population are tabulated in ***Table* I**.

Of the analyzed 120 children with asthma, 42.5% were labelled as responders. Comparison of responders and non-responders shows that they were are ell-matched in characteristics which could have confounded the response to salbutamol (***Table* I**).

No significant association was found between the *ADRB2* genotypes and salbutamol-responsiveness (*P*= 0.55) (***Table* II**). However, a trend towards greater bronchodilator responsiveness was seen amongst those who were carrying the A/A polymorphism as compared to G/G homozygotes. The median of change in percentage predicted FEV1 in the three *ADRB2* genotypes was 14.5 for A/A, 12.5 for A/G and 7.7 for G/G genotypes, respectively.

No significant association was found between the studied ADRB2 genotypes and the severity of asthma (*P*= 0.39) or family history of asthma (*P*=0.25). In the 120 children with asthma, the frequency of AA, GG, AG genotypes had prevalence of 24.2%, 24.2% and 51.7% respectively and were in equilibrium as per the Hardy Weinberg law (χ^2= 0.13).

Copyright of Indian Pediatrics 2016
For personal use only. Not for bulk copying or unauthorized posting to listserv/websites

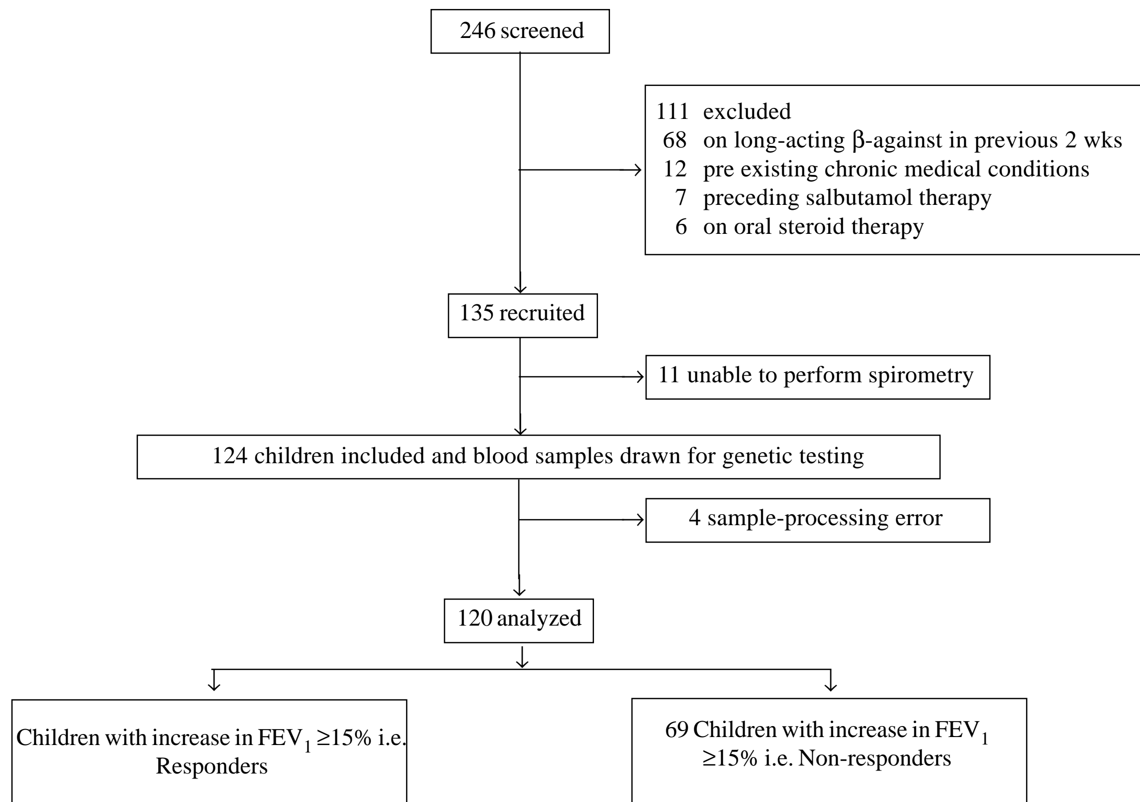

***Fig.* 1** *Study flow for screening, enrolment and stratification into responders and non-responders.*

TABLE I BASELINE CHARACTERSITICS OF RESPONDERS *VERSUS* NON RESPONDERS

Variable	*Responders (n=51)*	*Non-responders (n=69)*	*P value*
Age; mean (SD), y	9.3 (2.12)	9.1 (2.37)	0.76
Female gender, *n* (%)	11 (21.6)	18 (26.1)	0.56
Height; mean (SD), cm	131.3 (13.1)	131.0 (15.3)	0.90
Weight; mean (SD), kg	27.08 (8.47)	26.89 (10.1)	0.91
Age at onset; mean (SD), mo	46.11 (36.6)	55.95 (42.4)	0.19
Severity asthma, n (%)			0.85
Intermittent	06 (11.7)	09 (13.0)	
Mild persistent	26 (50.9)	30 (43.5)	
Moderate persistent	18 (35.3)	29 (42.0)	
Severe persistent	01 (01.9)	01 (01.4)	
Family history of allergy, *n* (%)	42 (60.9)	35 (68.6)	0.38

One hundred fourteen non-asthmatic adults were enrolled as controls to determine prevalence of *ADRB2* genotypes. Twenty samples were lost to processing errors, leaving 94 for analysis. Mean age of control population was 42.5 yrs (39.3% females). The prevalence of *ADRB2* genotypes was 20.2%, 59.6%, and 20.2% for A/A, A/G and G/G, respectively which fall within the Hardy Weinberg equilibrium.

TABLE II *ADRB2* GENOTYPE AND BRONCHODILATOR RESPONSE IN THE STUDY POPULATION

ADRB2 Genotype	*Responders no. (%), n = 51*	*Non-responders, no. (%), n = 69*
A/A	14 (27.4)	15 (21.7)
A/G	27 (52.9)	35 (50.7)
G/G	10 (19.6)	19 (27.5)

P values for comparisons between A/A vs G/G, A/G vsG/G, A/A vs A/G and G/G vs A/A + A/G were all >0.05.

No *ADRB2* genotype was found more prevalent in the asthmatic population compared to the non-asthmatics (P=0.51). The allele frequencies were found to be exactly equal at 50.0% for wild type A allele as well as the mutant G allele in both the asthmatic and non-asthmatic groups.

DISCUSSION

In this observational study done amongst children with

Copyright of Indian Pediatrics 2016
For personal use only. Not for bulk copying or unauthorized posting to listserv/websites

WHAT IS ALREADY KNOWN?

- Polymorphism of *ADRB2* gene on chromosome 5 may influence the salbutamol response in acute asthma exacerbation.

WHAT THIS STUDY ADDS?

- Salbutamol responsiveness showed no correlation with the studied *ADRB2* genotypes.

asthma aged 5 to 15 years with acute exacerbation of asthma, no association was found between the *ADRB2* genotype (Arg16Gly) and salbutamol-responsiveness defined as the percentage change in FEV_1 ≥15%.

Green, *et al*. [9] suggested that several SNPs of the *ADRB2* gene significantly alter *ADRB2* receptor down regulation. Unfortunately, the multitude of studies subsequently done have shown vastly discordant results. Early studies by Martinez, *et al.* [3] showed better bronchodilator response in asthmatics with A/A genotype of *ADRB2* compared to the G/G genotype (P=0.05). Later, similar results were seen in several studies [10-12]. A study by Choudhary, *et al.* [13] showed better salbutamol response in those with AA genotype in Puerto Ricans but not in Mexican highlighting ethnic differences. In contrast a few studies have shown absolutely opposite results [14]. The only Indian study done showed a better salbutamol response in those possessing the G/G *ADRB2* genotype [15]. Some larger studies have reported lack of any such association [16-20].

This study is one of the few studies which have researched the response to inhaled SABA during an acute exacerbation of asthma. Most other studies have focused on cohorts of stable asthma patients [3,10-12,15,16,18]. This has direct implications for finding the best personalized treatment for acute asthma attacks that are responsible for hospitalization and mortality. Percentage change in actual FEV was chosen as the main outcome measure as it is the most objective and immediate outcome. It has been taken as the study end-point in several studies, making the comparison of results easier. A suitably high cut-off of ≥15% was taken as a meaningful response to rule out any measurement variability [21]. Though no significant association could be established in the current study, a trend towards a greater positive bronchodilator response amongst the Arg16Arg homozygotes has been seen. A larger sample size could prove or disprove such an association. Importantly, the results of our study have not shown concordance with the only other Indian study on the subject [15], though it involved a Southern Indian population. This ethnic difference of the populations enrolled may explain the variability in the results obtained, highlighting the need of conducting these studies in various ethnic groups.

There are certain inherent limitations to our interpretations. The foremost is the fact that there are a multitude of SNPs of *ADRB2* gene and many of them may be in linkage disequilibrium, meaning that a certain set of alleles are more likely to be inherited together as a block. Thus the protective effect of one SNP may mask the adverse effect of another SNP when inherited together. Hence, research studying association of *ADRB2* haplotypes with bronchodilator response may be more relevant. The other concern is that extrapolation of these results to the Indian population warrants a larger sample size, with due importance given to the various ethnicities. Even though we have restricted our study to the northern Indian population, the region within itself contains diverse population groups requiring studies focussed on these subgroups. Thirdly, the study involved only two cases of severe persistent asthma which may possess altogether different genotypic and clinical manifestations compared to the less severe variants of asthma. Hence, more studies need to be conducted before drawing conclusions specifically in this group.

Contributors: PS: planning of study, data collection and analysis, laboratory work and writing of manuscript, SS: involved in planning of study, laboratory work and manuscript writing, RL: planning of study, data collection and analysis, and writing of manuscript, NG: data collection, writing of manuscript; RMP and SKK: planning of study, data analysis and writing of manuscript, MK: study planning, data collection, laboratory analysis, and manuscript writing. MK: will act as guarantor for the study.

Funding: None; *Competing interests*: None stated.

REFERENCES

1. Paramesh H. Epidemiology of asthma in India. Indian J Pediatr. 2002;69:309-12.
2. Drazen JM, Silverman EK, Lee TH. Heterogeneity of therapeutic responses in asthma. Br Med Bull. 2000;56:1054-70.
3. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between polymorphisms of ADRB2 and response to albuterol in children with and without history of wheezing. J Clin Invest. 1997;100:3184-88.
4. Hall IP, Blakey JD, Al Balushi KA, Wheatley A, Sayers I,

Copyright of Indian Pediatrics 2016
For personal use only. Not for bulk copying or unauthorized posting to listserv/websites

Pembrey ME, *et al*. Beta 2-adrenoceptor polymorphisms and asthma from childhood to middle age in the British 1958 birth cohort: a genetic association study. Lancet. 2006;368:771-9.

5. Lemeshow S, Hosmer DW Jr, Klar J, Lwanga SK. Tables for sample size determination, table 9h *In*: Lemeshow S, Hosmer DW Jr, Klar J, Lwanga SK. Adequacy of Sample Size in Health Studies. England, John Wiley & Sons Ltd, 1990. p. 156.
6. Carroll CL, Sekaran AK, Lere RJ, Schramm CM. A modified Pulmonary index score with predictive value for pediatric asthma exacerbations. Ann Allergy Asthma Immunol. 2005;94;355-9.
7. Sambrook J, Russell DW. Isolation of HMW DNA from mammalian cells using proteinase K and phenol, protocol 1. In: Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. Third edition; volume 1. New York, Cold Spring Harbor laboratory Press, 2001. p. 6.8.
8. Hartl DL, Clarke AG. Principles of Population Genetics. Sunderland, MA: Sinauer, 2007. p. 48-54.
9. Green SA, Cole G, Jacinto M, Innis M, Liggett SB. Polymorphism of human *ADRB2* alters functional properties of receptor. J Biol Chem. 1993;268:23116-21.
10. Kotani Y, Nishimura Y, Maeda H, Yokoyama M. Beta2-adrenergic receptor polymorphisms affect airway responsiveness to salbutamol in asthmatics. J Asthma. 1999;36:583-90.
11. Cho SH, Oh SY, Bahn JW, Choi JY, Chang YS, Kim YK, *et al*. Association between bronchodilating response to SABA and non-synonymous SNP of *ADRB2* gene. Clin Exp Allergy. 2005;35:1162-7.
12. Salah K, Morsy S, Atta A. Effects of *ADRB2* polymorphism on asthma severity and response to salbutamol on Egyptian children. Egypt J Pediatr Allergy Immunol. 2012;10:81-6.
13. Choudhry S, Ung N, Avila PC, Ziv E, Nazario S, Casal J. Pharmacogenetic differences in response to albuterol between Puerto Ricans and Mexicans with asthma. Am J Respir Crit Care Med. 2005;171:563-70.
14. Carroll CL, Stoltz P, Schramm CM, Zucker AR. *ADRB2* polymorphisms affect response to treatment in children with severe asthma exacerbations. Chest. 2009;135:1186-92.
15. Kukreti R, Bhatnagar P, B-Rao C, Gupta S, Madan B, Das C, *et al*. Beta(2)-adrenergic receptor polymorphisms and response to salbutamol among Indian asthmatics. Pharmacogenomics. 2005;6:399-410.
16. Tsai HJ, Shaikh N, Kho JY, Battle N, Naqvi M, Navarro D, *et al*. *ADRB2* polymorphisms: Pharmacogenetic response to bronchodilator among African American asthmatics. Hum Genet. 2006;119:547–57.
17. Martin AC, Zhang G, Rueter K, Khoo SK, Bizzintino J, Hayden CM, *et al*. *ADRB2* polymorphisms predict response to beta2-agonists in children with acute asthma. J Asthma. 2008;45:383-8.
18. Szczepankiewicz A, Breborowicz A, Sobkowiak P, Kramer L, Popiel A. Role of *ADRB2* gene polymorphism in asthma and response to beta2-agonists in Polish children. J Appl Genet. 2009;50:275-81.
19. Fu WP, Zhao ZH, Zhong L, Sun C, Fang LZ, Liu L, *et al*. Relationship between polymorphisms in the 5 leader cistron, positions 16 & 27 of ADRB2 gene & asthma in a Han population from southwest China. Respirology. 2011;16:1221-7.
20. Isaza C, Sepúlveda-Arias JC, Agudelo BI, Arciniegas W, Henao J, Porras GL, *et al*. ADRB2 polymorphisms in asthmatic and non-asthmatic school children from Colombia and their relationship to treatment response. Pediatric Pulmonol. 2012; 47:848-55.
21. Nickerson BG, Lemen RJ, Gerdes CB, Wegmann MJ, Robertson G. Within-subject variability and per cent change for significance of spirometry in normal subjects and in patients with cystic fibrosis. Am Rev Respir Dis. 1980;122:859-66.

Copyright of Indian Pediatrics 2016
For personal use only. Not for bulk copying or unauthorized posting to listserv/websites