Original Article

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Beta 2 Adrenoceptor Polymorphism and Response to Salbutamol in Asthmatics of Punjabi Origin in Islamabad

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Author`s Contribution

[1]Substantial contributions to the conception, design of the study;
[2]Interpretation of data for the work and final approval of the version to be published
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ABSTRACT

Objectives: To associate single nucleotide polymorphism of adrenoceptor beta2 gene (Arg16Gly rs 1042713) with bronchial asthma in patients of Punjabi ethnicity in Islamabad and to compare pharmacogenetic aspect by salbutamol responsiveness in asthmatics with and without single nucleotide polymorphism of adrenoceptor beta2 gene (Arg16Gly rs 1042713).

Methodology: This cross-sectional study was conducted in Physiology department of Al-Nafees Medical College & Hospital Islamabad in collaboration with Islamabad Diagnostic Center and COMSATS University Islamabad from January 2021 to June 2022. A total of 289 asthmatics and 289 non-asthmatics were enrolled. Genotype was done by Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR) method. Salbutamol responsiveness was done based on symptomatic improvement by bronchial Asthma Control Test (ACT) of Global Initiative for Asthma (GINA).

Results: Risk estimation calculations between asthmatics and non-asthmatics showed no association with polymorphism as depicted by nonsignificant chi square p value (0.9), odds ratio (95% confidence interval); OR (CI); 0.98 (0.70-1.33) and chi square p value (0.8), OR (CI); 1.0 (0.71-1.51) in dominant and recessive model respectively. Salbutamol responsiveness revealed strong association with AA genotype as reflected by highly significant chi square p value 0.0001, OR (CI); 7.5 (4.1-13.7) between AA vs AG and chi square p value 0.0001, OR (CI); 8.4 (4.2-16.8) between AA vs GG respectively.

Conclusions: There was no evidence of association of single nucleotide polymorphism of adrenoceptor beta2 gene (Arg16Gly rs 1042713) with bronchial asthma in Punjabi ethnics of Islamabad. Comparison of salbutamol responsiveness revealed AA homozygotes better responders than AG and GG carriers.

Key Words: Polymorphism, adrenoceptor beta2, Bronchial asthma, Salbutamol, Beta 2 Agonists, Bronchial Responsiveness.

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Introduction

Bronchial asthma is characterized by chronic inflammation of airways with involvement of many cells like mast cells, eosinophils and T lymphocytes which cause airways obstruction leading to wheeze, shortness of breath, chest tightness, and coughing. The condition can be reversible spontaneously or with treatment.[1]

Studies have reported that more than 300 million people have been affected by bronchial asthma worldwide with ascending order of occurrence. In Pakistan the figures vary in different cities with 25.68% prevalence in Islamabad.[2,3]

In bronchial asthma delivery of air to lungs is impaired in inflamed airways due to tripartite of adverse processes that include inflammatory edema of bronchial walls, constriction of airways and mucus plugging of airways.[3] Aside from the various environmental factors, different genetic factors are reported to be associated with susceptibility of bronchial asthma along with many other factors such as an individual's ethnicity/ race, gender, and geographical region.[4]

Beta 2 adrenoceptor (β_2AR) is transmembrane G protein coupled receptor (GPCR) which is densely presented on bronchial wall. β_2AR encoded by adrenoceptor beta2 gene (ADRB2) which is intron less gene localizes on locus 32 of long arm of chromosome 5.[5] ADRB2 gene an alteration at 46th position of nucleotide adenine to guanine leads to the change of amino acid at 16th position from arginine to glycine (AGA to GGA). Since the change occurs at DNA flanking site which presents the ligand binding site of the receptor, its alteration impairs ligands binding with receptor and attributes in bronchial asthma development and agonist response.[6]

Studies have reported mild bronchial asthma severity with Arg16Arg genotype.[7] Although G allele has been described in most studies as mutant allele and A allele as wild type, but Arg16Arg genotype showed beta 2 agonist receptor downregulation as compared to Gly16Gly homozygotes Arg16Gly heterozygotes. Data also reports lower frequencies of Gly16Gly in asthmatics.[8]

In Serbian children it has found that Arg16Gly polymorphism can predict bronchial asthma severity and altered response with salbutamol treatment.[6] Indian study found better bronchodilator response in Arg16Arg genotype with salbutamol.[9]

Since discordant and controversial results obtained from several studies. Some studies provide data to have association of asthma with ADRB2 variants and response with salbutamol in Arg16/Arg genotype better than Arg16/Gly and Gly16/Gly. Others provide contradistinction in results.[10,11]

To delineate and elucidate the findings in Pakistani study population we aimed to find out the frequencies of different genotypes of Arg16/Gly polymorphism in asthmatics and non-asthmatics with Punjabi ethnicity with age and gender match groups in Islamabad. Comparison of salbutamol responsiveness among different genotypes was included in objectives to find out the pharmacogenetic impact in implementation of therapy.

Methodology

A descriptive case series study was conducted in the This cross-sectional study was conducted in Al-Nafees Medical College & Hospital Islamabad in collaboration with Islamabad Diagnostic Center and COMSATS University Islamabad letter no F. 2/IUIC-ANMC/EC-225/2020. A total of 289 asthmatics of adult onset of moderate bronchial asthma and 289 controls both males and females with Punjabi ethnicity (having Punjabi parents) were enrolled by nonprobability consecutive sampling. Patients with respiratory diseases other than the bronchial asthma and allergic disorders, bronchial asthma with extreme severity, chronic medical illnesses, and use of long-acting beta agonists & steroid therapy in prior 4 weeks were excluded. Based on the observation of the frequency of Glycine homozygotes, which have been associated with greater β2ARs downregulation, and population estimation with a defined level of absolute precision, the sample was computed by the WHO calculator.[9]

The diagnosis of bronchial asthma of moderate severity was chosen and it was done with the GINA 2021 guidelines.[12] Anamnesis and physical examination were done in all participants in accordance with COVID 19 protocol.[13] However, salbutamol responsiveness was done on the basis bronchial asthma ACT questionnaire defined by GINA with salbutamol inhalation therapy. ACT comprised of five item version was used and cut off values of ≤19 predicted as controlled and uncontrolled bronchial asthma.[14] Asthmatics patients with moderate stable bronchial asthma were kept on salbutamol inhalation therapy as Ventolin Evohaler 200ug (2 puffs three times daily for 2 weeks preceded by 4 weeks run in period.[15]

Three ml of blood was drawn from ante-cubital vein from each participant and blood was stored at 4° C for analysis. The usual phenol-chloroform method was used to extract DNA, and the following procedures were taken.[16]

PCR-RFLP method was used for the targeted and allele-specific amplifications of the SNP in the ADRB2 gene were carried out. Utilizing the Ensemble Genome Browser and primer3.ut.ee for primers of RFLP-PCR that are allele-specific for the gene's polymorphism were used. Forward and Reverse Primer Sequence (5' to 3') was GCCTTCTTGCTGGCACCCCAT with 21 base pairs and CAGACGCTCGAACTTGGCCATG of 22 base pairs with16 hours digestion time by Ncol restriction

enzyme were synthesized and obtained. By using insilico-PCR, the forward and reverse primers' specificity was verified. After reconstitution of primers PCR analysis was done and its products were examined by gel documentation system.

A total volume of 30 µl was created by combining 10 µl of PCR product, 1 µl of restriction enzyme, 3 µl of 10 x buffers, and 16 µl of deionized water. Sixteen hours of incubation at 37 degrees Celsius were carried out on a shaking water bath, and the results were then examined on a 2.5% agarose gel. The ADRB2 rs 1042713 polymorphism was amplified using a spanning sequence of primers. The SNP was amplified in a segment of DNA. After PCR (168 bp), the Nocardia corallina (NcoI) registration enzyme was used to perform RFLP on the PCR results. The homozygous AA wild type allele was resolved at bands of 146 bp fragments, the 128 bp fragments for the GG mutant allele, and the 146 and 128 bp fragments for the AG heterozygous variants.

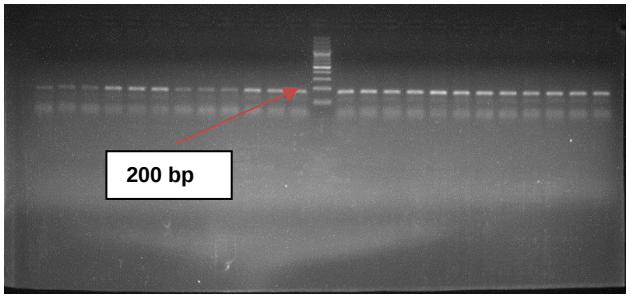

Figure 1. Gel Electrophoresis of PCR products.

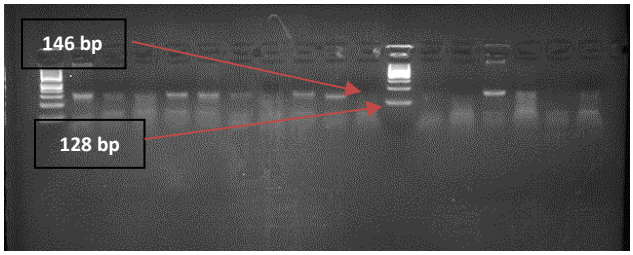

Figure 2. ADRB2 46 A>G rs 1042713 Detection by RFLP-PCR.

Statistical Package for Social Sciences (SPSS) Version 25 was used to apply the Shapiro-Wilk test for normality of the data and the median and interquartile ranges; median (IQR) for continuous demographic variables along with comparison by Mann Whitney U test. Chi square tests were used to evaluate categorical demographic characteristics. Determination of genotype and allele frequencies along with comparison of genotypes of good and bad responders were done by binary logistic regression.

Results

A total of 578 patients were inducted in the study after formal informed consent. Group I (n=289) control; non-asthmatics and group II (n=289) asthmatics. Demographic features of the study included age, gender and extrinsic & intrinsic asthma based on age of the onset of bronchial asthma symptoms along with family history of allergy. Both groups were age and gender matched as depicted by statically non-significant difference between groups. Table I. The frequencies of AA genotype and allele with wild type healthy allele A in both groups were comparable. Similarly genotype AG which possesses both healthy allele A and mutant also called risk allele G were more in numbers and percentages in both groups. Contrariwise GG genotype with mutant risk allele G has also been found to be with equal distribution in both groups but it was minor recessive allele in the current study. Aside from the decreased frequency of mutant risk allele G in asthmatics the results of binary logistic regression depict no association between occurrence of asthma and presence of mutant risk allele in the present study (Table II). It was found that alleles were consistent with Hardy Weinberg Equilibrium (HWB).

Table I: Demographic parameters comprised of comparison of age distribution between non-asthmatics and asthmatics.

Parameters	Control; non-Asthmatics (n = 289)	Asthmatics (n = 289)	p value
Age median (IQR)	45(39- 52)	45(41- 52)	0.80 (Mann Whitney U test)
Gender			0.46
Male	132 (45%)	130 (44%)	(Chi square
Female	157 (57%)	159 (56%)	test)
Asthma Type		144 (49%)	0.36
Extrinsic	---	145 (51%)	(Chi square
Intrinsic			test)

The dominant genetic model of genetic analysis was comprised of comparison of dominant homozygous AA genotype (AA vs AG + GG) and the recessive genetic model of genetic analysis was comprised of comparison of recessive homozygous GG genotype (GG vs AG + AA). Binary logistic regression was used in the comparison of both the genotypes and alleles which comprised of odds ratio (OR), relative risk (RR) with 95% Confidence Interval (CI), and Pearson's chi-square p values. Table II.

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Table II: Genetic Heterogeneity Analysis comprised of comparison of Genetic dominant model analysis, recessive model analysis and comparisons of genotypes with respect to good and bad response to salbutamol by Binary Logistic Regression comprised of Chi square p value, Odds ratio, and Relative risk with 95% confidence interval. G* (mutant allele).

Gene & SNP (rs)	Parameters	Control; non-Asthmatics n = 289 Genotypes & Alleles n (%)	Asthmatics n = 289 Genotypes & Alleles n (%)	p value (Chi square)	Odds Ratio (95% Cl)	Relative Risk (95% Cl)
ADRB2 Gene rs 1042713	Genotypes Dominant Model Analysis	AA = 108 (37%) AG = 111 (39%) GG = 70(24%) AA vs AG+GG 108 + 181	AA = 107 (36%) AG = 110 (38%) GG = 72(26%) AA vs AG+GG 107 + 182	0.9	0.98 (0.70-1.38)	0.99 (0.82-1.17)
	Alleles Dominant Model Analysis	A = 327(56%) G* = 251(44%) A 327 G 251	A = 324 (55%) G*= 254 (45%) A 324 G 254	0.8	0.97 (0.77-1.2)	0.99 (0.87-1.1)
	Genotypes Recessive Model Analysis	GG Vs AA+AG 70+219	GG Vs AA+AG 72+217	0.8	1.0 (0.71-1.5)	1.0 (0.82-1.2)
	Alleles Recessive Model Analysis	G 251 A 327	G 254 A 324	0.8	1.0 (0.80-1.2)	1.0 (0.89-1.1)
	Comparison of Genotypes					
	AA vs AG	79 30	28 80	0.0001	7.5 (4.1-13.7)	2.7 (2.0-3.8)
	AA vs GG	18 79	54 28	0.0001	8.4 (4.2-16.8)	2.8 (2.0-3.9)

Results of ACT accumulative bronchial asthma score was expressed as mean ± SD and cut off values for good and bad responders was ≤19. Figure 3

The bar graph presents the blue bars as good responders and red bars bad responders with n (%) in AA, AG and GG genotypes of group II asthmatics which depicted the percentage of good responders were highest in AA genotype and of bad responders in GG genotype. Fig ure 4.

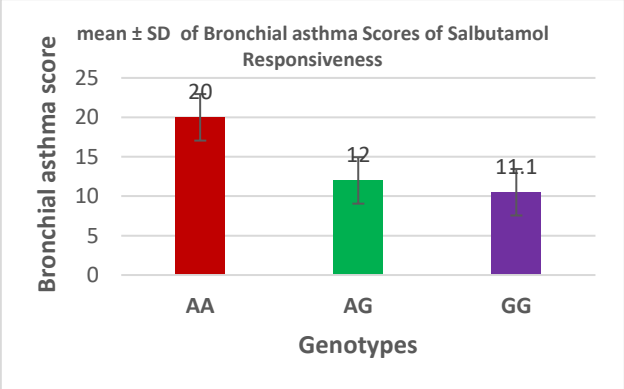

Figure 3. Accumulative Symptoms Score of Salbutamol Responsiveness in Asthmatics by Bronchial asthma Control Questionnaire (ACT) Global Initiative of Bronchial asthma Guidelines (GINA) 2021 p value by Kruskal Wallis Test.

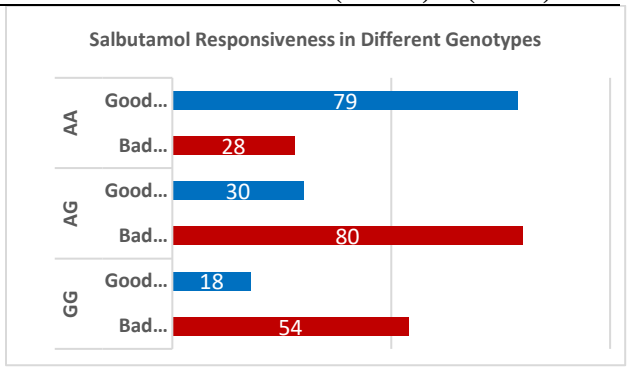

Figure 4. Frequencies (n %) of Good and Bad Responders in Genotypes of Group II Asthmatics.

Discussion

The parameters of demography as age and gender were matched between non-asthmatics and asthmatics as reflected by statistically non-significant difference between groups in the current study to minimize confounding.

The first part of genetic study was heterogeneity analysis which comprised of dominant and recessive genetic models. An Indian study conducted by Sahi PK *et al* found no association with bronchial asthma severity and with family history of bronchial asthma in all genotypes of ADRB2. Among genotypes, AG heterozygotes were more in frequency than others. RFLP-PCR method of

genotyping was used. The dominant genetic analysis revealed nonsignificant statistical results suggestive of findings that individuals with ADRB2 +46A>G allele have no risk for development of moderate bronchial asthma. Genotype frequencies were AA 24%, AG 52% and GG 24% in asthmatics and controls revealed AA 20%, AG 59%, and GG 21%. Epigenetics (environmental and behavioral factors) attribute to the lack of association as found in study population of the current study also. The data produced by the study are in correlation with that of the present study most likely due to closely allied geographic region, RFLP-PCR method, and similarity in other epigenetic factors also.[9]

Yan LP and colleagues obtained the similar results with age, gender, and asthma types matched groups in Chinese ethnics. These findings are in correlation with results of research studies in terms of no association depicted by nonsignificant chi square p value and OR less than one. The recessive genetic model showed the similar trend.[17]

Regarding association studies of ADRB2, N Jovicic *et al* included only asthmatics with selected Serbian ethnicity and compared two SNPs. Genotypes were AA24%, AG 35% and GG 40% whereas Arab ethnics revealed AA12%, AG 48% and GG 40% despite of similar method of genotyping with adequate group randomization and demographic data. Difference in ethnicity, sample size and genetic architecture could be attributable factors for different results.[6,18]

Data from research studies revealed that polymorphic variant of ADRB2 at codon 16 has been described as one most studied polymorphism despite heterogeneity in findings exists worldwide. A British cohort longitudinal study recruited 8018 asthmatics from age 7 to 45 years. Symptoms were ascertained by detailed anamnesis before age 35 followed assessment done by pulmonary functions tests. During follow up at age 45 DNA genotyping was done for analysis of variants of ADRB2; arg16gly. AA at codon 16 homozygotes being more frequent in study population showed strong association with bronchial asthma symptoms. Racial difference and method of genotyping as haplotype analysis could cause inconsistent results.[19]

Results of the second part of the current study revealed that individuals with AA genotype were better responders of salbutamol therapy as compared to those with AG and GG genotypes (OR 7.5 and 8.4 respectively). Research studies reported that GG homozygotes have been described as poorer responders to salbutamol therapy. Even though different route of administration of salbutamol the similar genetic makeup particularly those of south Indian ethnicity and closely allied region could be the probable cause of constant results.[20,21] These results are also consistent with those obtained by the studies as better response to salbutamol inhalation therapy was found in AA genotype than AG and GG.[22,23] The same drug response can occur due to the gene-gene and gene-environment interaction because ADRB2 gene affects the genes expression of enzymes which are responsible for drug metabolism.[24]

In African American decent a study by Martinez *et al* analyzed better salbutamol response with arg16 homozygotes but Tsai Hg *et al* discovered no association arg16gly polymorphism with spirometric response to bronchodilators. Unique culture, lifestyle and ethnic diversity could cause heterogeneity in results between these two studies and current study.[25]

The findings of current study concluded that SNP of ADRB2 (Arg16Gly rs 1042713) with moderate bronchial asthma in patients of Punjabi ethnics of Islamabad has not been linked/associated with development asthma per se but it does affect altered pharmacogenetic characteristics (receptor binding with natural ligands as catecholamines and beta 2 agonist salbutamol, pharmacokinetics and pharmacodynamics) as reflected by good response with healthy wild type allele and bad response with mutant risk allele.

Conclusion

There is no evidence of association of single nucleotide polymorphism of ADRB2 (Arg16Gly rs 1042713) with moderate bronchial asthma in patients of Punjabi ethnicity in Islamabad as reflected by non-significant difference of genotype frequencies of AA homozygotes wild type allele, GG homozygotes mutant allele and AG heterozygotes carriers in asthmatics and non-asthmatics. Comparison of pharmacogenetic aspect by salbutamol responsiveness in asthmatics with and without single nucleotide polymorphism revealed that AA homozygotes wild type allele were better responders than GG homozygotes mutant allele and AG heterozygotes in asthmatics. Findings are suggestive of opting for genotyped tailored bronchial asthma control therapy.

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