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No Correlation between Beta2-Adrenergic Receptor Polymorphisms and the Severity and Clinical Control of Geriatric Asthma and COPD

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Abstract

Background: Polymorphisms of the β2-adrenergic receptor (ADRB2) have previously been associated with non-specific bronchial hyper-responsiveness, adverse response to β2-agonists and variable effects on lung function. The objective of this study was to determine whether genotypic variance in ADRB2 polymorphisms in a cohort of geriatric men and women with asthma and/or COPD correlate with disease severity, baseline pulmonary function, and the ability to maintain clinical control of their disease.

Methods: This comparative, prospective cohort study sequenced two ADRB2 polymorphisms, Arg16 --> Gly and Gln27 --> Glu, in 103 geriatric patients with a clinical history of asthma and/or COPD. Primary endpoints included pulmonary exacerbation rate, hospitalization rate, and quality of life scores.

Results: Arg/Arg genotype comprised 13.6% of the cohort. No significant differences in baseline pulmonary functions were noted across genotypic variants. No significant difference was associated with genotype and change in lung function, exacerbations, clinical hospitalizations, exercise tolerance and subjective quality of life assessment over 6 months of follow-up.

Conclusion: We conclude that in a geriatric asthma and/or COPD population, ADRB2 polymorphisms are not a factor in the ability to control disease.

Keywords: Beta2-adrenergic receptor; Polymorphism; Geriatric; Asthma; COPD; Arg16Gly; Gln27Glu

Introduction

Asthma and Chronic Obstructive Pulmonary Disease (COPD) fall under the category of obstructive lung disease, both characterized physiologically by air flow obstruction. The obstruction in asthma is largely reversible whereas the airflow limitation in COPD tends to be persistent and not fully reversible [1,2]. Overlap between the two conditions exists characterized by airway limitation which does not fully remit in addition to symptoms of chronic bronchitis and emphysema. Similarly, COPD variants may be characterized with airway hyperactivity whose airflow obstruction is partially reversible [2].

Bronchodilators are the mainstay of treatment in both diseases. One type, beta2-agonists, stimulates the beta2-adrenergic receptor increasing cyclic AMP thereby inducing bronchial relaxation [2]. The other principle class of bronchodilators, anti-cholinergics, leads to broncho-dilation via a different mechanism by blocking cholinergic effect on muscarinic receptors [2].

In the last decade, the β_2-adrenergic receptor gene (ADRB2) has been implicated in asthma and obstructive lung disease therapeutic response [3]. ADRB2 is located on chromosome 5q31.32 and encodes the beta2-adrenergic receptor of which a number of Single Nucleotide Polymorphisms (SNPs) exist. Of these, two non-synonymous SNPs have been well characterized, occurring at codon 16 and 27. Position 16 is reflected by a substitution of arginine with glycine whereas at position 27, glutamate is replaced with glutamine.

ADRB2 polymorphism studies on therapeutic response, albuterol use, mortality and disease severity are well published particularly in the pediatric population. The results of such studies on disease risk and treatment response result in conflicting results [3-8]. Specific ADRB2 polymorphisms may be associated with COPD structural lung disease, disease control and drug specific response to therapy [9-14]. Variable ADRB2 study designs have yielded conflicting data in the past decade. Despite specific associations with obstructive lung disease, ADRB2 polymorphisms are not well studied in the geriatric population. Given the severity of obstructive lung disease in this growing demographic, our study sought to examine the role of ADRB2 gene polymorphisms on mixed asthma and COPD disease control in a geriatric population.

Methods

Study patients

This study was approved by the University of California Los Angeles, Wadsworth VA Institutional Review Board, and all participants signed informed consent before entering the study. Subjects were randomly enrolled into this comparative, prospective cohort study from a list of asthma or COPD patients who visited the West Los Angeles Veterans Affairs Medical Center Allergy and Immunology clinic. The diagnosis of asthma was made either by an allergist or a pulmonologist, and COPD was diagnosed according to the GOLD guidelines [2].

Enrolled patients were either male or female, >55 years of age, either current or ex-smokers with at least one year of clinical follow-up.

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None of the patients had a respiratory infection or an exacerbation for at least 6 months prior to the study. All patients received standard of care therapy based upon National Asthma Education and Prevention Program (NAEPP) and/or the Global Strategy for the Diagnosis, Management and Prevention of COPD (GOLD) guidelines [2,15]. Primary outcomes included frequency of exacerbations, hospitalization rates, and quality of life scores over 6 months of follow-up.

DNA genotyping

DNA was obtained from buccal swabs using a sodium hydroxide lysis method, modified as follows. A piece of swab was removed by forceps and soaked in 40 ml of 0.2 M NaOH at 75.8°C for 5 min. Neutralization was completed by adding 360 ml .04 M Tris, pH 7.5, for a total of 400 ml of genomic DNA. Five microliters of this solution was used as template for PCR amplification. ADRB2 sequences containing the polymorphic bases were amplified by PCR in a 25-ml reaction containing 1 PCR buffer, 1 U Taq DNA polymerase (Invitrogen, Carlsbad, CA), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and each primer at 400 mM. Primer sequences were determined as follows: (forward: 5′-GCGGCTTCTTCAGAGCAC-3′; reverse: 5′-CCAC-CCACCCACACCTCGTCCC-3′). PCR was amplified under the following conditions: 96.8°C for 5 min, followed by 30 cycles of denaturing (96.8°C for 30 sec), annealing (55.8°C for 30 sec), and extension (72.8°C for 30 sec), with a final extension at 72.8°C for 6 min. The ADRB2 genotype at codons 16 and 27 were determined from the PCR product using SNaPshot (Applied Biosystems, Inc., Foster City, CA). The genotyping primer sequence for codon 16 is 5′-AAAAAAACCTTCTTGCTGGCACCCAAT-3′ and for codon 27 is 5′-AAAAAAAAAAAAAACGGACCACGACGTCACGCA-3′.

Juniper score

The validated Juniper Score for Asthma was utilized every 3 months during a clinic visit [16]. The 32 question survey was completed by the patient. If the patient was not comfortable or literate to complete the evaluation, a staff member assisted by reading the questions. Research staff ensured completion of each survey prior to data analysis. The scores collected each month were evaluated as a mean (averaged over 3 months). The mean scores were also analyzed annually for quarterly visit variability.

COPD clinical questionnaire

The validated COPD clinical questionnaire was utilized every 3 months during a clinic visit [17,18]. The 10 question survey was completed by the patient. If the patient was not comfortable or literate to complete the evaluation, a staff member assisted by reading the questions. Research staff ensured completion of each survey prior to data analysis. The scores collected each month were evaluated as a mean (averaged over 3 months). The mean scores were also analyzed annually for quarterly visit variability.

Asthma/COPD staging

All patients underwent pulmonary function testing (body plethysmography) annually with spirometry measurements every 3 months. Patients were staged as intermittent, mild, moderate or severe asthma persistent based upon NAEPP asthma guidelines or COPD stage I-IV based upon GOLD criteria [2,15]. Clinical control of disease state is described in Tables 1 and 2.

Statistical analysis

Chi square and t-tests were used to examine potential differences in patient characteristics by genotypic variance. Impact of genotypic polymorphisms on percent of patients in control of asthma or COPD over total visits was examined and the significance determined by means of chi square tests. Cox regression analysis was applied to evaluate differences in the distribution of duration of clinical control of asthma or COPD vs. genotype subgroups. Significance was assessed by means of the Wald Chi Square test. Analyses were performed using SAS v 8.0.

	Mild Intermittent	Mild Persistent	Moderate Persistent	Severe Persistent
Symptoms	<2/week	>2/week	Daily	Continual
Night symptoms	<2/month	>2/month	>1/week	Frequent
FEV_1	>80% predicted	>80% predicted	60-80%	<60%
Peak flow variability	<20%	20-30%	>30%	>30%

Table 1: Classification of asthma severity [15].

Stage	Spirometry
GOLD 1: Mild	$FEV_1 \geq 80\%$ predicted
GOLD 2: Moderate	$50\% \leq FEV_1 < 80\%$ predicted
GOLD 3: Severe	$30\% \leq FEV_1 < 50\%$ predicted
GOLD 4: Very Severe	$FEV_1 < 30\%$ predicted

Table 2: COPD Staging [2].

	Frequency	Percent
Gender		
Female	15	14.56
Male	88	85.44
Race		
African American	17	16.50
Caucasian	72	69.90
Hispanic	11	10.68
Pacific Islander/Asian	3	2.91
Smoking Status		
Non-Smoker	92	89.32
Smoker	11	10.68

Table 3: Baseline demographics of the study participants.

Results

The study recruited 116 patients initially. Absent genotype records resulted in 103 enrollees, characterized in Table 3. Mean age was 68.7 with ages ranging between 55 and 92. The demographic, as expected from the VA population, was predominantly male, with 88 males enrolled in the study (85.4%). At codon 16, 35.9% (n=37) were homozygous for the major ADRB2 allele (Gly/Gly), 50.5% (n=52) were heterozygous (Arg/Gly), and 13.6% (n=14) were homozygous for the minor allele (Arg/Arg). At position 27, 32.0% (n=33) were homozygous for the major ADRB2 allele (Gln/Gln), 51.5% (n=53) were heterozygous (Gln/Glu), and 16.5% (n=17) were homozygous for the minor allele (Glu/Glu). The overall patient characteristics including asthma stage, COPD stage, number of exacerbations, and number of oral steroid versus haplotype are displayed in Table 4. Of note, no combinations of the Arg/Arg and Glu/Glu alleles enrolled in our study population.

Asthma and COPD staging

Specific polymorphisms and asthma severity staging are displayed in Table 5. No statistical difference in frequency was noted between ADRB2 polymorphism and asthma stage severity (p-value range 0.9708-0.9947). Specific polymorphisms and COPD severity staging

	Gln/Gln Arg/Arg	Gln/Gln Arg/Gly	Gln/Gln Gly/Gly	Gln/Glu Arg/Arg	Gln/Glu Arg/Gly	Gln/Glu Gly/Gly	Glu/Glu Arg/Gly	Glu/Glu Gly/Gly
Participants	**11**	**15**	**7**	**3**	**32**	**18**	**5**	**12**
Male	9 (81.82%)	14 (93.33%)	7 (100%)	3 (100%)	27 (84.38%)	13 (72.22%)	5 (100%)	10 (83.33%)
Asthma Stage								
1	3 (27.27%)	2 (13.13%)	0	0	5 (15.63%)	1 (5.56%)	0	2 (16.67%)
2	2 (18.18%)	2 (13.13%)	2 (28.57%)	0	6 (18.75%)	8 (44.44%)	3 (60%)	2 (16.67%)
3	5 (45.45%)	8 (53.33%)	4 (57.14%)	1 (33.33%)	16 (50%)	7 (38.39%)	1 (20%)	5 (41.67%)
4	1 (9.09%)	3 (20%)	1 (14.29%)	2 (66.67%)	5 (15.63%)	2 (11.11%)	1 (20%)	3 (25%)
COPD Stage								
1	4 (36.36%)	4 (26.67%)	0	0	9 (28.13%)	6 (33.33%)	2 (40%)	3 (25%)
2	3 (27.27%)	6 (40%)	4 (57.14%)	0	14 (43.75%)	6 (33.33%)	1 (20%)	5 (41.67%)
3	3 (27.27%)	4 (26.67%)	2 (28.57%)	2 (66.67%)	6 (18.75%)	5 (27.78%)	1 (20%)	2 (16.67%)
4	1 (9.09%)	1 (6.67%)	1 (14.29%)	1 (33.33%)	3 (9.38%)	1 (5.56%)	1 (20%)	2 (16.67%)
Exacerbation								
0	5 (45.45%)	8 (53.33%)	0	0	15 (46.88%)	3 (16.67%)	2 (40%)	5 (41.67%)
1	3 (27.27%)	3 (20%)	5 (71.43%)	2 (66.67%)	11 (34.38%)	13 (72.22%)	1 (20%)	3 (25%)
2	1 (9.09%)	2 (13.33%)	2 (28.57%)	0	4 (12.50%)	1 (5.56%)	1 (20%)	1 (8.33%)
3	0	1 (6.67%)	0	0	0	0	0	2 (16.67%)
4	1 (9.09%)	0	0	1 (33.33%)	1 (3.13%)	1 (5.56%)	0	0
5	0	0	0	0	1 (3.13%)	0	0	0
6	1 (9.09%)	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	1 (8.33%)
12	0	1 (6.67%)	0	0	0	0	1 (20%)	0
Oral Steroid								
0	5 (45.45%)	8 (53.33%)	1 (14.29%)	0	16 (50%)	4(22.22%)	2 (40%)	5 (41.67%)
1	3 (27.27%)	3 (20%)	4 (57.14%)	2 (66.67%)	12(37.5%)	11(61.11%)	1 (20%)	3 (25%)
2	1 (9.09%)	2 (13.13%)	1 (14.29%)	0	2 (6.25%)	2 (11.11%)	1 (20%)	1 (8.33%)
3	2 (18.18%)	0	0	0	0	0	0	2 (16.67%)
4	0	0	1 (14.29%)	0	1 (3.13%)	1 (5.56%)	0	0
5	0	1 (6.67%)	0	1 (33.33%)	1 (3.13%)	0	0	0
8	0	0	0	0	0	0	0	1 (8.33%)
13	0	0	0	0	0	0	1 (20%)	0
14	0	1 (6.67%)	0	0	0	0	0	0

Table 4: Statistical summary of the participants, Asthma stage, COPD stage, Exacerbation and Oral Steroid use by genotype (Arg16Gly and Gln27Glu combination).

are displayed in Table 6. No statistical difference in frequency was noted between ADRB2 polymorphism and COPD stage severity (p-value range 0.6843-0.9434). Figures 1 and 2 displays the distribution of specific polymorphisms versus asthma or COPD staging and do not show a significant frequency difference among different genotype groups.

Juniper and COPD clinical questionnaire score

No statistical difference in frequency was noted between ADRB2 polymorphism and mean Juniper score (p-value=0.747). Similarly, no statistical difference in frequency was noted between ADRB2 polymorphisms and mean COPD Clinical Questionnaire (p-value range 0.845). Sub-analysis of mean Juniper and COPD Clinical Questionnaire variability during quarterly visits also did not correlate with ADRB2 polymorphism (p-value>0.05).

Exacerbations and hospitalizations

No statistical difference in frequency of exacerbation was noted between ADRB2 polymorphism (p-value range 0.1319-0.0561). Of note, five patients deemed steroid dependent were excluded from this analysis. No statistical difference in frequency of hospitalization was noted between ADRB2 polymorphisms (p-value range 0.132-0.447).

Discussion

Asthma and COPD disease modification are focused in recent years on genetic mutations, epigenetics and post-translational modification. The ADRB2 polymorphism remains an area of focus stemming from initial links to asthma morbidity and mortality [19-22]. Associations with specific ADRB2 polymorphisms may predict response to long acting beta agonists (LABA), inhaled corticosteroids (ICS) and anticholinergics [23,24]. Additionally, the COPD population, including geriatric patients, may be susceptible to such predilection based upon recent studies [25]. Alternatively, a number of studies refute such associations [3,26-28]. Given the discrepancy in management required if ADRB2 polymorphisms are implicated, our study uniquely studied the clinical effect of ADRB2 polymorphisms in the most progressive population of obstructive lung disease, geriatrics.

Across all genotypic ADRB2 variants, no statistically significant

		Stage 1[a]	Stage 2	Stage 3	Stage 4	Total
Codon 16 polymorphism	**Arg/Arg**					
	Frequency	3	2	6	3	14
	Percent[b]	2.91%	1.94%	5.83%	2.91%	13.59%
	Arg/Gly					
	Frequency	7	11	25	9	52
	Percent	6.80%	10.68%	24.27%	8.74%	50.49%
	Gly/Gly					
	Frequency	3	12	16	6	37
	Percent	2.91%	11.65%	15.53%	5.83%	35.92%
	Total					
	Frequency	13	25	47	18	103
	Percent	12.62%	24.27%	45.63%	17.48%	100.00%
Codon 27 polymorphism	**Gln/Gln**					
	Frequency	5	6	17	5	33
	Percent	4.85%	5.83%	16.50%	4.85%	32.04%
	Gln/Glu					
	Frequency	6	14	24	9	53
	Percent	5.83%	13.59%	23.30%	8.74%	51.46%
	Glu/Glu					
	Frequency	2	5	6	4	17
	Percent	1.94%	4.85%	5.83%	3.88%	16.50%
	Total					
	Frequency	13	25	47	18	103
	Percent	12.62%	24.27%	45.63%	17.48%	100.00%

[a]Stage 1-4 corresponds to mild intermittent, mild persistent, moderate persistent, and severe persistent

[b]Derived from frequency divided by total number of subjects (103). e.g., Arg/Arg Stage 1: 3/103 = 2.91%

Table 5: Distribution of codon 16 and codon 27 polymorphisms by asthma stage.

differences in baseline pulmonary function were noted. Standardized objective clinical outcomes in lung disease included both objective criteria of staging, disease variability, hospitalizations, and exacerbation rate. No statistically significant difference was observed with objective clinical outcomes and ADRB2 polymorphisms. Similarly, the subjective validation of clinical asthma and COPD was studied utilizing validated survey tools. Again, no statistically significant difference was noted between the Juniper Score for asthma and the COPD Clinical Questionnaire score for COPD and ADRB2 polymorphisms.

The results of our study are supported by prior authors in both genetic and clinical disease state. Migita and colleagues demonstrated the presence of ADRB2 polymorphisms did not increase the risk of asthma development in families [8]. Similarly, a lack of Arg 16 allele and Gln 27 allele polymorphism frequency was noted in two COPD cohorts, Vacca et al. and Joos et al. [11,29]. A meta-analysis performed by Niu and colleagues examining twelve case-control studies and eight cross-sectional studies further confirmed polymorphisms of ADRB2 at positions 16 and 27 did not change the risk of COPD [30]. Functional studies support the modifying role of ADRB2 polymorphisms in response to β2-agonists, β2-receptor down-regulation, and potential for disease severity [31-33]. Despite purported risk of specific polymorphisms, numerous studies have refuted such findings. Bleecker et al. revealed patients with ADRB2 polymorphisms showed equal response clinically to salmeterol, fluticasone, budesonide and formoterol in asthmatics [3,5]. Yelensky and colleagues noted similar findings in COPD patients treated with indacaterol [26]. Overall, our study demonstrated no clinical risk differential between ADRB2 genotypes and treatment of COPD and asthma in a geriatric population. Given the severity and progression of obstructive lung disease in this patient population, our findings suggest the role of ADRB2 polymorphisms in adult COPD and asthma is minimal. Nonetheless, specific, isolated clinical cases may be present in younger populations.

There are some limitations to the present study. Our study results were based on a small sample size and a predominantly male population. Though determination of clinical control was measured through the primary endpoints of number of exacerbations, hospitalization rates, and quality of life questionnaire scores, change in FEV_1 (delta FEV_1) was briefly reviewed and showed no association between genotype and change in lung function. Finally, we did not examine specific treatment regimens in the recruited patients. The relationship between ADRB2 polymorphism and different treatment regimens has been well studied during the last decade with mixed results [3-6,10,24-28]. As such, there has been no major paradigm shift in treatment of asthma or COPD with respect to varying ADRB2 polymorphisms. Considering the prevalence of asthma and COPD is highest in the geriatric population, the goal of this study was to determine the association between markers of asthma and COPD severity with ADRB2 polymorphisms in a cohort of patients 55 years and older.

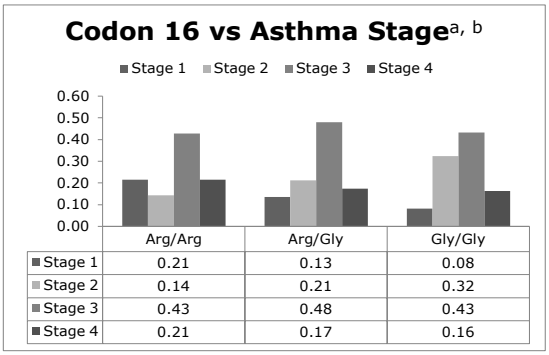
In conclusion, our findings demonstrate beta2-adrenergic receptor polymorphisms do not correlate with disease severity and clinical

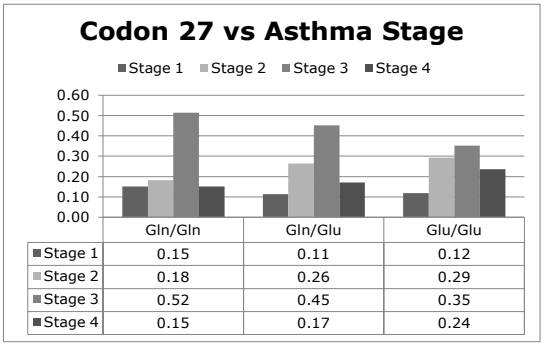
		Stage 1[a]	Stage 2	Stage 3	Stage 4	Total
Codon 16 polymorphism	**Arg/Arg**					
	Frequency	4	3	5	2	14
	Percent[b]	3.88%	2.91%	4.85%	1.94%	13.59%
	Arg/Gly					
	Frequency	15	21	11	5	52
	Percent	14.56%	20.39%	10.68%	4.85%	50.49%
	Gly/Gly					
	Frequency	9	15	9	4	37
	Percent	8.74%	14.56%	8.74%	3.88%	35.92%
	Total					
	Frequency	28	39	25	11	103
	Percent	27.18%	37.86%	24.27%	10.68%	100.00%
Codon 27 polymorphism	**Gln/Gln**					
	Frequency	8	13	9	3	33
	Percent	7.77%	12.62%	8.74%	2.91%	32.04%
	Gln/Glu					
	Frequency	15	20	13	5	53
	Percent	14.56%	19.42%	12.62%	4.85%	51.46%
	Glu/Glu					
	Frequency	5	6	3	3	17
	Percent	4.85%	5.83%	2.91%	2.91%	16.50%
	Total					
	Frequency	28	39	25	11	103
	Percent	27.18%	37.86%	24.27%	10.68%	100.00%

[a]Stage 1-4 corresponds to GOLD COPD Stage I-IV

[b]Derived from frequency divided by total number of subjects (103). e.g., Arg/Arg Stage 1: 4/103=3.88%

Table 6: Distribution of codon 16 and codon 27 polymorphisms by COPD stage.

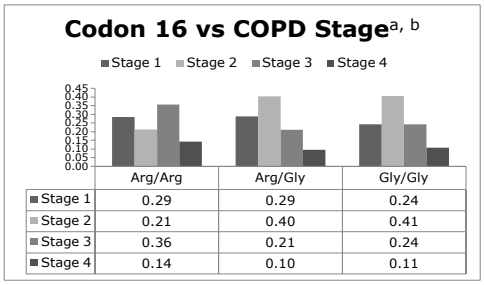


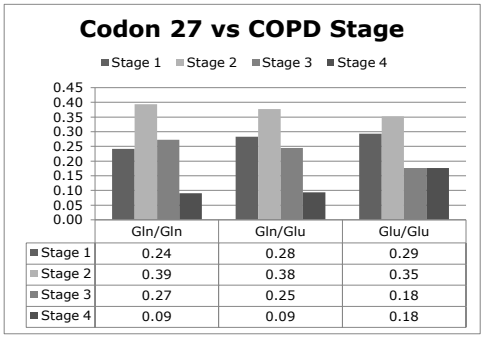


[a]Stage 1-4 corresponds to mild intermittent, mild persistent, moderate persistent, and severe persistent

[b]Numerical values derived from total patients within an asthma stage with a certain genotype divided by total subjects with that genotype. e.g., Arg/Arg Stage 1: 3 Arg/Arg subjects classified as Stage 1 out of a total of 14 subjects with the Arg/Arg genotype.

Figure 1: Distribution of different genotype groups by Asthma Stage.





[a]Stage 1-4 corresponds to GOLD COPD Stage I-IV

[b]Numerical values derived from total patients within a COPD stage with a certain genotype divided by total subjects with that genotype. e.g., Arg/Arg Stage 1: 4 Arg/Arg subjects classified as Stage 1 out of a total of 14 subjects with the Arg/Arg genotype.

Figure 2: Distribution of different genotype groups by COPD Stage.

control of geriatric patients with asthma and COPD. However, we did not have a significant number of active smoker patients, 11 patients (10.68%). Additionally, we did not assess second hand smoke exposure in our patient population. Environmental exposures such as smoking or second hand smoking are known epigenetic modifiers via DNA methylation [34-35]. Epigenetic changes can additionally be affected by diet and aging [36-37]. A recent study, examining twins using high resolution computer tomography (HRCT), also demonstrated lung parenchymal and small airway changes were associated with a heritable background [38]. The immune system, cellular function, and phenotype of asthma/COPD may change with age and indeed affect ADRB2 function. Further research is needed to study the epigenetic effects in asthma and COPD. Hence, as further epigenetics on aging, cellular immunity, regulatory T cell (T_{reg}) function and lung epithelia are discovered, the conclusion of no effect may indeed be incorrect.

Authors' Contributions

WK along with IR designed the research study and collected and analyzed data. AP, IR, and JY drafted the manuscript. All authors read and approved the final manuscript.

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