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Beta$_2$-Adrenergic Receptor Gene Haplotypes in Chronic Obstructive Pulmonary Disease

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List of Abbreviations

χ^2	Chi-square test
AC	Adenylyl cyclase
ADRB2	$Beta_2$-adrenergic receptor gene
ANOVA	Analysis of variance
Arg16Gly	Amino acid substitution from arginine to glycine at codon 16
BDR	Bronchodilator response
BHR	Bronchial hyper-responsiveness
BMI	Body mass index
Ca^{2+}	calcium ion
cAMP	Cyclic adenosine monophosphate
CI	Confidence interval
CO	Carbon monoxide
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
DALYs	Disability-adjusted life years
DE	Echocardiography with Doppler imaging
DL	Diffusing capacity
DLCO	Carbon monoxide gas transfer capacity
DNA	Deoxyribonucleic acid
ENaC	Epithelial sodium channels
FEV_1	Forced expiratory volume after one second
FVC	Forced vital capacity
GDP	Guanosine diphosphate
GERK2	G protein–coupled receptor kinases
Gi	Inhibitory G protein
Gln27Glu	Amino acid substitution from glutamine to glutamic acid at codon 27
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GR	Glucocorticoid receptor

Gs	Stimulatory heterotrimeric G-protein
GTP	Guanosine triphosphate
HRCT	High-resolution computer tomography
HWE	Hardy-Weinberg equilibrium
ICS	Inhaled corticosteroids
ICU	Intensive care unit
JVP	Jugular venous pressure
KCO	Carbon monoxide gas transfer coefficient
KD	Kilodaltons
LABA	Long-acting $beta_2$-agonist
LD	Linkage disequilibrium
LHS	Lung Health Study
MAF	Minor allele frequencies
MAPK	Mitogen-activated protein kinase
MMRC	Modified Medical Research Council dyspnea scale
NCGC	National Clinical Guideline Centre
NIMV	Non-invasive mechanical ventilation
NIPPV	Noninvasive positive-pressure ventilation
Nt	Nucleotide
OR	Odds ratio
PCR	Polymerase chain reaction
PDE	Phosphodiesterase
PEFR	Peaked expiratory flow rate
PFT	Pulmonary function test
PKA	Protein kinase A
RV	Right ventricular
SABA	Short-acting $beta_2$-agonist
SDS	Software-Defined Storage
SNPs	Single nucleotide polymorphisms
SPSS	Statistical Package for the Social Sciences
UTR	untranslated regions
WHO	World Health Organization

β_2-agonists	Beta$_2$-adrenergic agonists
β_2-AR	Beta$_2$-adrenergic receptor
βARK	β-adrenergic receptor kinase

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INTRODUCTION AND RATIONALE

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide (***Zhang et al., 2014***). It has been estimated that COPD occurs in 4-10% of the population, but further increase in its prevalence can be predicted in the coming decades (***Pauwels and Rabe 2004; Lacoma et al., 2009; Berndt et al., 2012***). COPD is currently the fourth leading cause of death in the world, and is projected to be the third by 2020 (***Halvani et al., 2007; Moatassem et al., 2010; Papatheodorou et al., 2010***).

COPD is characterized by the presence of an airflow limitation that is not fully reversible, and is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases (***Lacoma et al., 2009; Brashier and Kodgule, 2012***). Beta$_2$-adrenergic agonists (β_2-agonists) are the most widely bronchodilators used to treat airflow limitations associated with COPD. Inter-individual variation in therapeutic responses to β_2-agonists can be determined by several factors, including the degree of baseline airflow limitation, smoking status, age, and genetic factors (***Hizawa et al., 2007; Al-Rubaish, 2011; Karam et al., 2013***).

The reversibility of airflow limitations in response to a bronchodilator is an important component of COPD. Therefore, the bronchodilator response (BDR) may be a useful indicator, not only for assessing the clinical effect of treatment for COPD, but also for predicting the clinical outcome and survival (***Hizawa et al., 2007***).

COPD represents a complex-trait disease with contributions of multiple genes and environmental risk factors, especially smoking (***Joos et al., 2002; Demeo et al., 2006; Marson et al., 2012***). The beta$_2$-adrenergic receptor (β_2-AR) gene (*ADRB2*) is located on chromosome 5q31-32, a region that is genetically linked with functional changes in the β_2-AR in the respiratory system (***Kim et al., 2009; Al-Rubaish, 2011***).

The β_2-AR mediates the physiologic responses of the airways, including bronchodilation, anti-inflammatory actions, mucociliary clearance, and vascular endothelial permeability (***Hizawa et al., 2007; Yang et al., 2007; Augusto et al., 2010;***

Marson et al., 2012). Alterations in the *ADRB2* gene have a significant physiologic role in regulating responses to exogenous β_2-agonists, and may also affect the signaling and function of other receptors that control airway contractility such as cholinergic receptors (***Hizawa et al., 2007***).

ADRB2 is a small gene without introns, consists of a single exon of 2015 nucleotides, which encodes a 413 amino acid protein (***Augusto et al., 2010; Karam et al., 2013***). This gene has several natural genetic variants (polymorphisms).The most frequent non-synonymous single nucleotide polymorphisms (SNPs) occur in the coding region within the extracellular amino terminus of the receptor. The first SNP is the change of adenine to guanine (A > G) at nucleotide 46 causes amino acid substitution from arginine to glycine at codon 16 (Arg16Gly; *rs1042713*). The second SNP is the change of cytosine to guanine (C > G) at nucleotide 79 results in the substitution from glutamine to glutamic acid at codon 27 (Gln27Glu; *rs1042714*) (***Hesse and Eisenach, 2008; Augusto et al., 2010; Al-Rubaish, 2011***; ***de Paiva et al., 2014***).

These polymorphisms have functional consequence on the adrenergic receptors in vascular and bronchial smooth muscle (***Fukui et al., 2006; Leineweber and Heusch, 2009; Augusto et al., 2010***). The possible clinical relevance of these coding SNP in *ADRB2* has been explored in a range of disorders including respiratory illness, congestive heart failure, coronary heart disease, hypertension, cystic fibrosis, obesity, diabetes, colorectal carcinoma, Graves' disease, rheumatoid arthritis, atopic dermatitis, open-angle glaucoma, and polycystic ovary syndrome (***Gope et al., 2005; Maxwell et al., 2005; Inagaki et al., 2006; Kurabayashi et al., 2006; Roguedaset al., 2006; Chu et al., 2009***).

Strong linkage disequilibrium (LD) exists between these SNPs, resulting in common combination of polymorphisms (haplotypes): Glu_{27} is almost always associated with Gly_{16}, whereas Gln_{27} is associated with either Arg_{16} or Gly_{16} (***Leineweber and Heusch, 2009; Karam et al., 2013***). Significant difference in the distributions of these genotypes and haplotype structure of *ADRB2* gene were noted in different ethnic populations (***Hegab et al., 2004; Maxwell et al., 2005; Hesse and***

Eisenach, 2008). This genetic difference may explain some of the variability in disease manifestations and responses to treatment to the β_2-agonists in patients with COPD (***Xie et al., 1999; Hizawa et al., 2007; Al-Rubaish, 2011; Karam et al., 2013***).

Only scarce data with inconsistent results are available on the role of the *ADRB2* genotype in patients with COPD. In addition, data on allelic variation at this site among Egyptian patients with COPD are limited. Thus, this study was needed to unravel the potential importance of the *ADRB2* genotype as a marker for COPD susceptibility, and to know which polymorphism may influence disease prognosis and response to treatment among Egyptian patients.

AIM OF THE STUDY

The purpose of this study is to assess the genetic association between beta-2 adrenergic receptor (*ADRB2*) gene polymorphisms and chronic obstructive pulmonary disease among Egyptian patients.

STUDY QUESTIONS

1. Is the allelic frequency of *ADRB2* gene polymorphism different between COPD patients and healthy individuals?
2. Are *ADRB2* genetic variants correlated with COPD phenotypes, especially pulmonary function, and the bronchodilator response (BDR) to short-acting $beta_2$-agonist (SABA)?

HYPOTHESIS

The allelic frequency of *ADRB2* gene in COPD patients is different from that in healthy individuals, and is associated with the clinical outcome of the disease as well as the BDR to SABA.

OBJECTIVES OF THE STUDY

The objectives of the current study were

Primary objectives

1. To determine the allele frequency of *ADRB2* gene polymorphisms among Egyptian population.
2. To evaluate if variations within *ADRB2* gene polymorphisms as genotypes or haplotypes confer an increased prevalence of COPD among Egyptians.
3. To investigate the association between *ADRB2* gene polymorphisms and the severity of COPD.
4. To investigate the relationship between *ADRB2* gene polymorphisms and the differential responses to short-term BDR in COPD patients.

Secondary objectives

1. To assess the association of *ADRB2* gene polymorphisms and pulmonary function parameters.

Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is a global health concern, and is a major cause of chronic morbidity and mortality worldwide, and imparts a substantial economic burden on individuals and society. According to the World Health Organization (WHO), it is currently the sixth leading cause of death in the world, and further increases in the prevalence and mortality of the disease is predicted for the coming decades (***WHO, 2011; El-Zein, 2012***).

Definition of COPD

Several lung societies have provided practice guidelines and position statements in an attempt to define COPD. According to the GOLD (global initiative for chronic obstructive lung disease), chronic obstructive pulmonary disease, a common preventable and treatable disease, is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients (***GOLD, 2014***).

The chronic airflow limitation characteristic of COPD is caused by a mixture of small airways disease (obstructive bronchiolitis) and parenchymal destruction (emphysema), the relative contributions of which vary from person to person (***GOLD, 2014***). According to National Clinical Guideline Centre (NCGC) guidelines, COPD is characterized by airflow obstruction. The airflow obstruction is usually progressive, not fully reversible and does not change markedly over several months. The disease is predominantly caused by smoking (***NCGC, 2010***).

Epidemiology of COPD

COPD prevalence, morbidity and mortality vary appreciably across countries and across different groups within countries but generally are directly related to prevalence of tobacco smoking (***Bakke et al., 2011***).

Prevalence of COPD

The burden of COPD is underestimated worldwide because it is not usually recognized and diagnosed until it is clinically apparent and moderately advanced. It has been estimated that COPD occurs in 4-20% of the population, but further increase in its prevalence can be predicted in the coming decades. Large differences exist between countries. These are attributable to many factors, including differences in diagnostic methods, year of study, age of the population, and prevalence of main risk factors such as tobacco smoking (***Pauwels & Rabe 2004; Lacoma et al., 2009***).

Recent prevalence, morbidity, and mortality in Egypt are still lacking and have to be estimated. In Egypt, in 1997, the prevalence of COPD was 23.1% in an elderly population living in a rural area in Minya Governorate (***Gomaa, 2004***). The prevalence of COPD increases with age, with a five-fold increased risk for those aged over 65 years compared with patients aged less than 40 years. The prevalence of COPD increases with smoking status (by a factor of five). COPD affects twice as many males as females but this difference will diminish, given the fact that more and more females throughout the world have taken up smoking in the past few years in developed countries, and that nonsmoking females are exposed to biomass combustion products in developing countries (***Raherison and Girodet, 2009***).

Impact of COPD on morbidity

In diagnosed patients, COPD is a disabling disorder which is accompanied by a negative impact on quality of life. The factors significantly associated with a poor quality of life are: severity of respiratory damage, estimated by Forced expiratory volume after one second (FEV_1); the use of oxygen treatment; the frequency of visits to the emergency room; and the frequency of hospitalization (***Raherison and Girodet, 2009; Gupta et al., 2013***). COPD is the leading cause of hospital admissions, particularly in older populations, with average 10-days length of stay. Hospitalization rates for COPD increase with age and are higher among men than among women. COPD also has a much higher readmission rate than other chronic illnesses as cardiovascular disease and diabetes (***Viegi et al., 2007***).

In addition, the weight of comorbidities which are frequently associated with COPD is important to consider. It is estimated that approximately two-thirds of patients with COPD have one or two comorbidities. The main comorbidities present are cardiovascular disorders, bronchial cancer, lung infections, thromboembolic disorders, the existence of associated asthma, high blood pressure, osteoporosis, joint pain, gastroduodenal ulcer, depression and anxiety (***Raherison and Girodet, 2009***).

Mortality attributable to COPD

According to the World Health Organization, COPD is the fourth leading cause of death in the world, with approximately 2.75 million deaths per annum, or 4.8% of deaths. Deaths due to or associated with COPD have been increasing steadily over the past 20 years, and it is expected to become the third leading cause of death worldwide by 2030. In Egypt, the mortality rate is approximately 40 deaths per 100,000 of population (***WHO, 2011***). Mortality from COPD is higher in males and increases with age in those over 45 years old. The death rate among men has reached a steady plateau, but the rate among women has still in continuous increase. Several factors have been found to be predictive of mortality in patients with COPD: severity of airflow obstruction, nutritional status (body mass index), exercise capacity using the 6-min walk test and severity of dyspnea (the BODE index) (***Viegi et al., 2007***).

The mortality rate during hospitalization for exacerbation of COPD is estimated to be 2.5–10%. The mortality rate after hospitalization varies between 16% and 19% in the 3 months following hospitalization, between 23% and 43% at 1 year and is 55–60% at 5 years. After classifying patients by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage, mortality rate at 5 years was 17% for stage I, 42% for stage II, 49% for stage III and 73% for stage IV. The only factors having demonstrated a reduction in mortality in COPD are cessation of smoking and oxygen treatment for patients with chronic respiratory failure (***Raherison and Girodet, 2009***).

Economic burden

The direct costs of COPD are the value of healthcare resources devoted to diagnosis and medical management of the disease. Indirect costs reflect the monetary consequences of

disability, missed work, premature mortality and caregiver or family costs resulting from the illness (***Sullivan et al, 2000; Gupta et al., 2013***). In COPD, the direct medical costs, which are the value of healthcare resources devoted to diagnosis and medical management of the disease, are substantial because the disease is both chronic and highly prevalent. It is estimated in 1993 of $14.7 billion (in US dollars). The estimated indirect costs related to morbidity (*ie*, loss of work time and productivity), premature mortality, and caregiver or family costs resulting from the illness were an additional $9.2 billion, for a total of $23.9 billion (***Halpin and Miravitlles, 2006***).

Social burden

COPD is a low-profile, insidious illness that proceeds almost imperceptibly, gradually depriving individuals of their health and vitality. It frequently affects people in their most productive years (***Faulkner et al., 2006***). In COPD, the disability-adjusted life years (DALYs), which are the sum of years lost because of premature mortality and years of life lived with disability, adjusted for the severity of disability, was the twelfth leading cause of DALYs lost in the world in 1990, the eighth leading cause of DALYs among men and the seventh leading cause of DALYs among women in 1996, and expected to be the fifth leading cause of DALYs lost worldwide in 2020, behind ischemic heart disease, major depression, traffic accidents, and cerebrovascular disease (***Mannino, 2002***). This substantial increase in the global burden of COPD projected over the next twenty years reflects, in large part, the increasing use of tobacco worldwide and the changing age structure of populations in developing countries (***Mannino and Buist, 2007***).

Risk factors of COPD

The identification of risk factors is an important step toward developing strategies for prevention and treatment on any disease. COPD results from a gene-environment interaction. Among people with the same smoking history, not all will develop COPD due to differences in genetic predisposition to the disease. Risk factors for COPD may also be related in more complex ways (***GOLD, 2014***).

Factors that influence disease development and progression

Table (1). Candidate genes for developing COPD (*Babusyte et al., 2006; Smolonska et al., 2009*).

Gene class	Gene
Protease & Antiprotease genes	α_1 Antitrypsin
	Tissue inhibitors of metalloproteinases (TIMPs)
	Matrix metalloproteinases (MMPs 1, 2, 9 and 12)
	α_1 Antichymotrypsin
	α_2 Macroglobulin
Antioxidant and xenobiotic metabolizing enzyme genes	Heme oxygenase-1
	Microsomal epoxide hydrolase
	Glutathione-S-transferase
	Cytochrome P-450 1A1
Inflammatory mediator genes	Tumor necrosis factor α
	Vitamin D-binding protein
	Interleuken-1α,-1β
	Interleukin-1 receptor, type 1
	Interleukin -1 receptor, type 2
	Intrerleuken-13
	Interleuken-1 receptor antagonist
Genes involved in airway defense	Human leukocyte antigens
	Cystic fibrosis transmembrane conductance regulator
	Defensins (α-defensin-1, -2)
	Surfactant proteins

(A) Host factors

1) *Genes*

The genetic risk factor that is best documented is a severe hereditary deficiency of alpha-1 antitrypsin (a major circulating inhibitor of serum proteases). Genetic association studies have implicated a variety of genes in COPD pathogenesis and lung function, Table

(1). Many of these genes are thought to be involved in inflammation, and therefore related to potential pathogenic mechanisms of COPD (***El-Zein, 2012; Cho et al., 2014***).

2) *Age and gender*

Age is often listed as a risk factor for COPD. It is unclear if healthy aging as such leads to COPD or if age reflects the sum of cumulative exposures throughout life. Most studies showed the higher prevalence of COPD among men more than women and higher mortality rates as well. But recently, this is no more quite accurate. As reflecting the changing patterns of tobacco smoking, data now show that the prevalence of the disease is now equal in men and women, (***Amer, 2010; Sorheim et al., 2010; GOLD, 2014***).

3) *Lung growth and development*

Lung growth is related to processes occurring during gestation, birth, and exposures during childhood and adolescence. Reduced maximal attained lung function (as measured by spirometry) may identify individuals who are at increased risk for the development of COPD. Factors that affect lung growth during gestation and childhood have the potential for increasing the individual's risk of developing COPD (***Stern et al., 2007; GOLD, 2014***).

4) *Asthma/Bronchial Hyperreactivity*

Asthma and airway hyper-responsiveness, identified as risk factor that contribute to the development of COPD are complex disorders related to a number of genetic and environmental factors. How these trends are related to the development of COPD is unknown, however, airway hyper-responsiveness may also develop after exposure to tobacco smoke or other environmental insults and this may be a result of smoking – related airway disease (***Brashier and Kodgule, 2012***).

(B) Exposures

Of the many inhalational exposures that people may encounter over a lifetime, only tobacco smoke and occupational dusts and chemicals are known to cause COPD on their own. Tobacco smoke and occupational exposures also appear to act additively to increase a person's risk of developing COPD (***Gupta et al., 2013***).

1) *Tobacco Smoke*

Cigarette smoking is by far the most important risk factor for COPD and most important way that tobacco contributes to risk of COPD. Cigarette smokers have a higher prevalence of respiratory symptoms and lung function abnormalities, a greater annual rate of decline in FEV_1, and greater COPD mortality rate than nonsmokers. Also pipe and cigar smokers have greater COPD morbidity and mortality rates than nonsmokers, although their rates are lower than those for cigarette smokers, other types of tobacco smoking popular in various countries are also risk factors for COPD (***Raad et al., 2011; Castaldi et al., 2014***).

Age at starting to smoke, total pack-years smoked, and current smoking status are predictive of COPD mortality. Not all smokers develop clinically significant COPD, which suggests that genetic factors must modify each individual risk (***GOLD, 2014***). Passive exposure to cigarette smoke (also known as environmental tobacco smoke or ETS) may also contribute to respiratory symptoms and COPD by increasing the lung's total burden of inhaled particles and gases. Smoking during pregnancy may also pose a risk for the fetus, by affecting lung growth and development in utero and possibly priming of the immune system (***Brashier and Kodgule, 2012***).

2) *Occupational Dusts and Chemicals*

Occupational dusts and chemicals (vapors, irritants, and fumes) can also cause COPD when the exposures are sufficiently intense or prolonged, such as those experienced by miners in many countries. Exposure to coal dust alone in sufficient doses can produce airflow limitation. Exposure to particulate matter, irritants, organic dusts, and sensitizing agents can cause an increase in airway hyper-responsiveness, especially in airways already damaged by other occupational exposures, cigarette smoke, or asthma (***Trupin et al., 2003***).

3) *Air pollution*

The potentially harmful effect of air pollution on lung function is understandably a subject of great concern. Despite intense investigative efforts over the past several decades, there remains a very incomplete understanding of the overall impact that air pollution has on

human health and the more specifically of the development of lung disease (***Ezzati et al., 2005***).

The role of outdoor air pollution in causing COPD is unclear and appears to be small when compared with that of cigarette smoking. Although it has also been difficult to assess the effects of single pollutants in long-term exposure to atmospheric pollution, air pollution from fossil fuel combustion, primarily from motor vehicle emissions in cities, is associated with decrements of respiratory function. The relative effects of short-term, high-peak exposures and long-term, low-level exposures are yet to be resolved (***GOLD, 2014***).

Reported respiratory symptoms, but not lung function deficiencies, have been associated with indoor nitrogen dioxide levels and damp housing .The use of various solid fuels for cooking without adequate ventilation may result in high levels of indoor pollution. Although the issue is not settled, available evidence suggests that air pollution might be a factor in the development of COPD (***Torres-Duque et al., 2008***).

4) *Recurrent infections*

A history of severe childhood infection has been associated with reduced lung function and increased respiratory symptoms in adulthood. Whooping cough, bronchiolitis or pneumonia in the first year of life were associated with a significant reduction in FEV_1 measured in the first decade (***Brashier and Kodgule, 2012; Gupta et al., 2013***).

5) *Socioeconomic Status, diet and nutrition*

There is evidence that the risk of developing COPD is inversely related to socioeconomic status, independent of factors as tobacco smoking and occupational exposure. Certain fatty acids that affect arachidonic acid metabolism, such as linoleic acid, may worsen pulmonary function, whereas intake of others (such as fish oils) may be protective. Also antioxidant vitamins (including C and E) may provide a protective benefit as reported by certain studies (***De Batlle et al., 2012; GOLD, 2014***).

Pathology and pathogenesis of COPD

Pathogenesis of COPD

Pathogenic triad: oxidative stress, protease–antiprotease imbalance, and inflammation, are important contributing factors in the pathogenesis of COPD (***Wood and Stockley, 2006; Arinir et al., 2009; Fischer et al., 2011***).

1. ***Oxidative stress***

Oxidative stress may be an important amplifying mechanism in COPD. Biomarkers of oxidative stress (e.g., hydrogen peroxide, 8-isoprostane) are increased in the exhaled breath condensate, sputum, and systemic circulation of COPD patients. Oxidative stress is further increased in exacerbations. Oxidants are generated by cigarette smoke and other inhaled particulates, and released from activated inflammatory cells such as macrophages and neutrophils. There may also be a reduction in endogenous antioxidants in COPD patients as a result of reduction in nuclear factor erythroid 2-related factor (Nrf2) that regulates many antioxidant genes (***Lin and Thomas, 2010; Fischer et al., 2011; GOLD, 2014***).

2. ***Protease-antiprotease imbalance***

There is compelling evidence for an imbalance in the lungs of COPD patients between proteases that break down connective tissue components and antiproteases that protect against this. Several proteases, derived from inflammatory cells and epithelial cells, are increased in COPD patients. There is increasing evidence that they may interact with each other. Protease-mediated destruction of elastin, a major connective tissue component in lung parenchyma, is believed to be an important feature of emphysema and is likely to be irreversible (***MacNee, 2007; Arinir et al., 2009; GOLD, 2014***).

3. ***Inflammation***

COPD is characterized by chronic inflammation throughout the airways, parenchyma and pulmonary vasculature. There is an increase in neutrophils, macrophages, and T lymphocytes (especially CD8) in various parts of the lung, and sometimesan increase in eosinophils in some patients, particularly during exacerbations. The intensity, cellular and molecular characteristics of the inflammation vary as the disease progresses. Activated

inflammatory cells release a wide variety of inflammatory mediators in COPD patients that attract inflammatory cells from the circulation (chemotactic factors), amplify the inflammatory process (pro-inflammatory cytokines), and induce structural changes (growth factors) (***Barbu et al., 2011; Yawn, 2012***).

Pathology of COPD

COPD comprises pathological changes in four different compartments of the lungs (central airways, peripheral airways, lung parenchyma and pulmonary vasculature), which are variably present in individuals with the disease (***Saetta et al., 2006; GOLD, 2014***).

- ***Central airways***

While bronchial glands hypertrophy and goblet cell metaplasia occur, airway wall changes include squamous metaplasia of the airway epithelium, loss of cilia, ciliary dysfunction, and increased smooth muscle and connective tissue (***Rodriguez-Roisin and MacNee, 2006***).

- ***Peripheral airways***

Bronchiolitis is present in the peripheral airways at an early stage of the disease. As the disease progresses, there is fibrosis and increased deposition of collagen in the airway walls (***Rodriguez-Roisin and MacNee, 2006; Gupta et al., 2013***).

- ***Lung parenchyma***

Emphysema is defined as an abnormal enlargement of air spaces distal to the terminal bronchioles, occurs in the lung parenchyma in COPD. As a result of emphysema there is a significant loss of alveolar attachments, which contributes to peripheral airway collapse (***Turatoa et al., 2001***).

- ***Pulmonary vasculature***

Pulmonary vascular changes begin early during the course of the disease. Initially; these changes are characterized by thickening of the vessel wall and endothelial dysfunction. These are followed by increased vascular smooth muscle and infiltration of the vessel wall by inflammatory cells. In advanced stages of the disease, there is collagen deposition and emphysematous destruction of the capillary bed. Eventually, these structural

changes lead to pulmonary hypertension and right ventricular dysfunction (cor-pulmonale) ***(Rodriguez-Roisin and MacNee, 2006).***

Pathophysiology of COPD

Pathological changes in COPD lead to corresponding physiological abnormalities that usually become evident first on exercise and later also at rest. Physiological changes characteristic of the disease include mucus hyper-secretion, ciliary dysfunction, airflow limitation, pulmonary hyperinflation, gas exchange abnormalities, pulmonary hypertension and cor pulmonale. They usually develop in this order over the course of the disease (***Brashier and Kodgule, 2012; GOLD, 2014***).

- ***Airflow limitation and pulmonary hyperinflation***

Expiratory airflow limitation is the hallmark physiological change of COPD. It is primarily irreversible with a small reversible component. The irreversible component of airflow limitation is primarily due to remodeling fibrosis and narrowing – of the small airways that produces fixed airways obstruction, and a consequent increase in airways resistance. The sites of airflow limitation in COPD are the smaller conducting airways including bronchi and bronchioles less than 2 mm in internal diameter (***Rodriguez-Roisin and MacNee, 2006; Yawn, 2012***).

Parenchymal destruction (emphysema) plays a smaller role in this irreversible component but contributes to expiratory airflow limitation and increase in airways resistance in several ways. Destruction of alveolar attachments inhibits the ability of the small airways to maintain patency. Alveolar destruction is also associated with a loss of elastic recoil of the lung, which decreases the intra-alveolar pressure during exhalation (***O'Donnell et al., 2001***).

Airway smooth muscle contraction, ongoing airway inflammation and intraluminal accumulation of mucus and plasma exudates may be responsible for the small part of airflow limitation that is reversible with treatment. Inflammation and accumulation of mucus and exudates may be particularly important during exacerbations (***GOLD, 2014***).

- ***Mucus hyper-secretion and ciliary dysfunction***

Mucus hyper-secretion in COPD is caused by the stimulation of the enlarged mucus secreting glands and increased number of goblet cells by inflammatory mediators such as leukotrienes, proteinases and neuropeptides. Ciliated epithelial cells undergo squamous metaplasia leading to impairment in mucociliary clearance mechanisms (***Turatoa et al., 2001; Hurst et al., 2010; Oldenburge et al., 2012; Ramos et al., 2014***).

- ***Gas exchange abnormalities***

These occur in advanced disease and are characterized by arterial hypoxaemia with or without hypercapnia. An abnormal distribution of ventilation:perfusion ratios—due to the anatomical changes found in COPD—is the main mechanism for abnormal gas exchange. The extent of impairment of diffusing capacity for carbon monoxide per litre of alveolar volume correlates well with the severity of emphysema (***MacNee, 2006***).

- ***Pulmonary hypertension and cor pulmonale***

This develops late in COPD, at the time of severe gas exchange abnormalities. Contributing factors include pulmonary arterial constriction (as a result of hypoxia), endothelial dysfunction, remodeling of the pulmonary arteries (smooth muscle hypertrophy and hyperplasia), and destruction of the pulmonary capillary bed. Structural changes in the pulmonary arterioles result in persistent pulmonary hypertension and right ventricular hypertrophy or enlargement and dysfunction (cor pulmonale) (***MacNee, 2006; Yawn, 2012***).

- ***Systemic Effects***

Systemic inflammation and skeletal muscle wasting contribute to limiting the exercise capacity of patients and worsen the prognosis irrespective of degree of airflow obstruction. Patients also have an increased risk of cardiovascular disease, depression and anxiety, cachexia, normocytic normochromic anemia, secondary polycythemia, and osteoporosis (***MacNee, 2006; Fabbri and Rabe, 2007, Rabinovich and MacNee, 2011***).

Pathology and pathophysiology of exacerbations

The progressive course of COPD is complicated by exacerbations that have many causes and occur with increasing frequency as the disease progresses. Mild COPD

exacerbations are associated with increase of both neutrophils and eosinophils in sputum and biopsies, while severe COPD exacerbations are associated with an increase in sputum neutrophils and eosinophils, at least in sputum. Exacerbations can be caused by infection (bacterial or viral), air pollution, and changes in ambient temperature (***MacNee, 2006***).

In mild exacerbations, airflow obstruction is unchanged or only slightly increased. Severe exacerbations are associated with worsening of pulmonary gas exchange due to increased inequality between ventilation and perfusion and subsequent respiratory muscle fatigue. The worsening ventilation-perfusion relation results from airway inflammation, edema, mucous hypersecretion, and bronchoconstriction. These reduce ventilation and cause hypoxic vasoconstriction of pulmonary arterioles, which in turn impairs perfusion (***MacNee, 2006; O'Donnell and Parker, 2006; Lai and Rogers, 2010***).

Respiratory muscle fatigue and alveolar hypoventilation can contribute to hypoxaemia, hypercapnia, and respiratory acidosis, and lead to severe respiratory failure and death. Hypoxia and respiratory acidosis can induce pulmonary vasoconstriction, which increases the load on the right ventricle and, together with renal and hormonal changes, results in peripheral edema (***MacNee, 2006; Yawn, 2012***).

Diagnosis and assessment of COPD

A clinical diagnosis of COPD should be considered in any patient who has dyspnea, chronic cough or sputum production, and/or a history of exposure to one or more of risk factors for the disease. The diagnosis should be confirmed by spirometry. The presence of a post bronchodilator $FEV_1/FVC < 0.70$ and $FEV_1 < 80\%$ predicted confirms the presence of airflow limitation that is not fully reversible (***GOLD, 2014***).

Medical history

COPD is heterogeneous in its presentation. The characteristic symptoms of COPD are chronic and progressive dyspnea, cough, and sputum production. Chronic cough and sputum production may precede the development of airflow limitation by many years.

Conversely, significant airflow limitation may develop without chronic cough and sputum production (***Mannino, 2002; Rabe et al., 2007***).

a) Cough

Initially, the cough may be intermittent, but later is present every day, often throughout the day. Cough syncope or cough rib fractures may occur **(*GOLD, 2014*).**

b) Sputum production

COPD patients commonly raise small quantities of tenacious sputum after coughing bouts. However, purulent sputum reflects the presence of acute exacerbation (***Mannino, 2002; Gupta et al., 2013***).

c) Dyspnea

The hallmark symptom of COPD is the reason most patients seek medical attention and is a major cause of disability and anxiety associated with the disease. Breathlessness in COPD is characteristically persistent and progressive. In patients with severe disease, dyspnea may be influenced by posture: being worse on standing or sitting than when they are supine (platypnea). Others, however, may have orthopnea. It was also postulated that dyspnea is a better prognostic indicator in assessment of COPD (***Han et al., 2010; Yawn, 2012***).

d) Wheezing and chest tightness

These are relatively non-specific symptoms as wheezes and chest tightness which may vary between days and over the course of a single day, however, absence of chest tightness does not exclude COPD diagnosis (***Rabe et al., 2007; GOLD, 2014***).

e) Others

COPD has been increasing recognized as a systemic illness, with effects on nutritional status, muscle wasting, and depression. Fatigue, weight loss and anorexia are common problems in patients with severe and very severe COPD. Unexplained weight loss is important because, if caused by COPD, it heralds a poor prognosis ***(GOLD, 2014).*** Past history of asthma, allergy, respiratory infections in childhood or any other respiratory diseases such as tuberculosis may be present. There may be family history of COPD or other respiratory disease. There might be history of exacerbations or previous hospitalizations for respiratory disorder (***Rabe et al., 2007; GOLD, 2014***).

Comorbidities, such as heart disease, osteoporosis, musculoskeletal disorders, malignancies, or neurological comorbidities that share the same risk factor (i.e. cigarette smoke exposure) may coexist. In addition, history of patient's exposure to risk factors, such as smoking, or occupational or environmental noxious agents, should be noted (***Rabinovich and MacNee, 2011; Akgün et al., 2012; Brashier and Kodgule, 2012***).

Physical Examination

In COPD, physical signs of airflow limitation are usually not present until significant impairment of lung function has occurred, and their detection has a relatively low sensitivity and specificity. A number of physical signs may be present in COPD, but their absence does not exclude the diagnosis (***GOLD, 2014***).

a) ***Combined inspection and palpation***

The chest examination may reveal signs of pulmonary hyperinflation such as the barrel shape, more horizontally set ribs, prominent sternal angle and wide subcostal angle. Resting respiratory rate is often more than 20 per minute and breathing can be relatively shallow. Signs of respiratory distress may exist as pursed-lip breathing, intercostal indrawing, and use of accessory muscles of respiration (***Jindal et al., 2004; Rabe et al., 2007***).

b) ***Percussion***

Chest percussion reveals findings of hyperinflation with obliteration of cardiac dullness and downward displaced upper border of liver dullness. Elsewhere, the note is hyperresonant ***(Jindal et al., 2004***).

c) ***Auscultation***

COPD patients often have breath sounds with prolonged expiratory phase and uniformly diminished intensity. Fine inspiratory crepitations and rhonchi are commonly heard. Auscultation of the heart may show signs of cor pulmonale, such as split of second sound (pulmonic) and murmurs of pulmonary or tricuspid insufficiency (***Kresten and Chapman, 1993***).

d) ***Systemic signs***

Neck vein distension, liver enlargement and peripheral edema could be due to cor pulmonale or severe hyperinflation. Loss of muscle mass and peripheral muscle weakness

are consistent with malnutrition and /or skeletal muscle dysfunction. Cyanosis or bluish color of the mucosal membranes may indicate hypoxemia (***Rabe et al., 2007; Brashier and Kodgule, 2012; GOLD, 2014***).

e) *Presence of complications*

The physical findings may change in the presence of complications. Almost all cases of COPD will progress to chronic cor pulmonale in due course of time. It is detected from the presence of signs suggestive of pulmonary hypertension and right ventricular enlargement and/or failure, such as a loud second heart sound, parasternal heave and raised jugular venous pressure (JVP) (***Garvey and Ortiz, 2012***).

Acute exacerbations of COPD are characterized by an abrupt increase of symptoms. Exacerbations are caused by bronchial infections in most instances. COPD patients suffer several episodes of acute exacerbation a year, often leading to increased hospitalizations, respiratory failure and even death (***Garvey and Ortiz, 2012***). Chronic respiratory failure can result from disease progression. It is suspected from the presence of tachypnea, cyanosis, flapping tremors, and altered sensorium. Other complications of COPD include pneumonia, polycythemia, and pneumothorax are also common ***(Jindal et al., 2004***).

Investigations

A. Spirometry

Spirometry is considered to be essential for the diagnosis of COPD, being a “gold standard” to quantitate airway obstruction (***Jing et al., 2009***). FVC is the maximal volume of air exhaled with maximally forced effort from a maximal inspiration, i.e. vital capacity performed with a maximally forced expiratory effort, expressed in liters at body temperature and ambient pressure saturated with water vapor (BTPS). FEV_1 is the maximal volume of air exhaled in the first second of a forced expiration from a position of full inspiration, expressed in liters at BTPS (***Miller et al., 2005***).

1) Measuring airflow limitation:

After taking a maximally deep breath, the subject exhales as forcefully as possible, and the volume of air exhaled is measured as a function of time. A reduction in the FEV_1/FVC

ratio below a cut-off point of 0.7 is diagnostic of obstruction (***GOLD, 2014***). Because it may take some time for patients to empty their lungs fully, particularly if COPD is present, the FEV after 6 seconds (FEV_6) can be used as a valid alternative for FEV_1/FVC in the diagnosis of airway obstruction (***Jing et al., 2009***). Interpretation of pulmonary function test (PFT) is based on comparisons with reference (predicted) values of normal subjects with the same anthropometric (e.g. sex, age and height), and ethnic characteristics of the patient being tested (***Pellergino et al., 2005***).

2) Reversibility tests:

☐ *Bronchodilator reversibility testing:*

Formed only once, at the time of diagnosis, this test is useful for several reasons; to help rule out a diagnosis of asthma. If FEV_1 returns to the predicted normal ranges after administration of a bronchodilator; to establish a patient's best attainable lung function at that point time; and to gauge a patient's prognosis. However, and despite earlier hopes, neither bronchodilator nor oral glucocorticosteroid reversibility testing predicts disease progression, whether judged by decline in FEV_1, deterioration of health status, or frequency of exacerbations in patients with a clinical diagnosis of COPD and abnormal spirometry. In some cases (e.g., a patient with an atypical history such as asthma in childhood and regular night waking with cough or wheeze), a clinician may wish to perform a bronchodilator and/or glucocorticosteroid reversibility test (***Rabe et al., 2007***).

☐ *Glucocorticosteroid reversibility testing:*

Long-term glucocorticosteroid treatment in COPD can at present only be justified in patients with consistent, significant FEV_1 response to glucocorticosteroids, or in those with repeated exacerbations. The simplest and potentially safest way to identify these patients is by a treatment trial with inhaled glucocorticosteroids for 6 weeks to 3 months. There is glucocorticosteroid reversibility if there is increase in the FEV_1 (200 ml and 12% above base line). The response to glucocorticosteroids should be evaluated with respect to the post-bronchodilator FEV_1. This should be performed at least once to exclude asthma and to establish the best lung function for the individual patient, and, to a lesser degree, estimate

the prognosis. The increase in FEV_1 should be expressed as a percentage of the predicted value that is less dependent on the baseline FEV_1. (***Gibson and McNee, 2006***).

B. Additional investigations:

1) Imaging:

Chest X-ray should be performed in all patients. It is not sensitive for the diagnosis in COPD, but it is valuable in excluding alternative diagnoses and establishing the presence of significant comorbidities such as concomitant respiratory (pulmonary fibrosis, bronchiectasis, pleural diseases, bullous disease), skeletal (e.g., kyphoscoliosis), and cardiac diseases (e.g., cardiomegaly). Radiological changes associated with COPD include signs of lung hyperinflation (flattened diaphragm, and an increase in the volume of the retrosternal air space), hyperlucency of the lungs, and rapid tapering of the vascular markings (***Rabe et al., 2007***).

Computed tomography (CT) of the chest is not routinely recommended. However, when there is doubt about the diagnosis of COPD, CT scanning might help in the differential diagnosis where concomitant diseases are present. In addition, if a surgical procedure such as bullectomy or lung volume reduction is contemplated, a chest CT scan is necessary since the distribution of emphysema is one of the most important determinants of surgical suitability (***Bafadhel et al., 2011; GOLD, 2014***).

2) Gas transfer:

Carbon monoxide gas transfer capacity (DLCO) and coefficient (KCO) are both reduced in symptomatic patients with COPD. The transfer coefficient is a good indicator of the presence and severity of emphysema and thus reduction in the KCO is helpful in distinguishing asthma from emphysema ***(GOLD, 2014).***

3) Static Lung volumes and Diffusing Capacity:

COPD patients exhibit gas trapping (a rise in residual volume) from early in the disease, and as airflow limitation worsens static hyperinflation (an increase in total lung capacity) occurs. These changes can be documented by body plethysmography, or less accurately by helium dilution lung volume measurement. These measurements help

characterize the severity of COPD but are not essential to patient management. Measurement of diffusing capacity (DL) provides information on the functional impact of emphysema in COPD and is often helpful in patients with breathlessness that may seem out of proportion with the carbon monoxide (CO) degree of airflow limitation (***Gibson and MacNee, 2006; GOLD, 2014***).

4) Oximetry and arterial blood gas measurement:

Pulse oximetry can be used to evaluate a patient's oxygen saturation and need for supplemental oxygen therapy. Pulse oximetry should be used to assess all stable patients with FEV_1 < 35% predicted or with clinical signs suggestive of respiratory failure or right heart failure. If peripheral saturation is < 92% arterial blood gases should be assessed (***GOLD, 2014***). Blood gas abnormalities worsen during acute exacerbations and may also worsen during exercise and sleep. The relationship between impaired spirometric values and blood gas abnormalities is weak; hypercapnia is observed with increasing frequency with the FEV_1 less than 1 liter (***Celli and MacNee, 2004; Gibson and MacNee, 2006***).

5) Exercise testing:

Objectively measured exercise impairment is a powerful indicator of health status impairment and predictor of prognosis. Walking tests can be useful for assessing disability and are used to assess the effectiveness of pulmonary rehabilitation. Both the paced shuttle walk tests and the unpaced 6-minute walk test can be used. Laboratory testing using cycle or treadmill ergometry can identify co-existing or alternative conditions, e.g., cardiac diagnoses. Monitoring of physical activity may be more relevant regarding prognosis than evaluating exercise capacity. This can be done using accelerometers or multisensor instruments (***Gibson and MacNee, 2006***).

6) Echocardiography:

Echocardiography with Doppler imaging (DE) can provide an estimated right ventricular (RV) systolic pressure, which is believed to reflect pulmonary artery systolic pressure in the absence of RV outflow tract obstruction (***Minai et al., 2010***). Still, the gold

standard method of measuring pulmonary hypertension remains right heart catheterization. ***(Janda et al., 2011)***

7) Alpha one antitrypsin serum level:

The WHO recommends that COPD patients from areas with a particularly high prevalence of alpha-1 antitrypsin deficiency should be screened for this genetic disorder. However, the typical patient tends to present at a younger age (< 45 years) with lower lobe emphysema. Thus it should be measured in young patients (4th or 5th decade) who develop COPD and have a strong family history. This may be followed by family screening. A serum level of α_1-antitrypsin <15–20% of the normal value is highly suggestive of homozygous α_1-antitrypsin deficiency ***(Rabe et al., 2007; GOLD, 2014)***.

8) Excercise testing:

It could be tested in poor nutrition and if suspect steroid myopathy, as well as if dyspnea or hypercapnia are disproportionally increased with respect to FEV_1. Maximum inspiratory pressure is impaired usually due to hyperinflation and/or abnormal mechanics of breathing. In contrast, a reduction in maximum expiratory pressure could be attributed to muscle weakness ***(GOLD, 2014).***

Differential diagnosis

Table (2). Differential diagnosis of COPD (*GOLD, 2014).*

Diagnosis	Suggestive Features
COPD	Onset in mid-life. Symptoms slowly progressive. History of tobacco smoking or exposure to other type of smoke.
Asthma	Onset early in life (often childhood). Symptoms vary from day to day. Symptoms at night/early morning. Allergy, rhinitis, and/or eczema also present. Family history of asthma.

Congestive heart failure	Chest X-ray shows dilated heart, pulmonary edema. Pulmonary function tests indicated volume restriction, not airflow limitation.
Brochiectasis	Large volumes of purulent sputum. Commonly associated with bacterial infection. Chest X-ray/CT shows bronchial dilation, bronchial wall thickening.
Tuberculosis	Onset all age. Chest X-ray shows lung infiltrate. Microbiological confirmation. High local prevalence of tuberculosis.
Obliterative bronchiolitis	Onset in younger age, nonsmokers. May have history of rheumatoid arthritis or acute fume exposure. Seen after lung or bone marrow transplantation CT on expiration shows hypodense areas.
Diffuse Panbronchiolitis	Predominantly seen in patients of Asian descent. Most patients are male and nonsmokers. Almost all have chronic sinusitis. Chest X-ray and HRCT show diffuse small centrilobular nodular opacities and hyperinflation.

Management and Prevention of COPD

Therapeutic Options:

An effective COPD management plan includes four components: (*1*) assess and monitor disease, (*2*) reduce risk factors, (*3*) manage stable COPD, and (*4*) manage exacerbations. Patients should be identified as early in the course of the disease as possible, and certainly before the end stage of the illness when disability is substantial. Once COPD has been diagnosed, effective management should be aimed to relieve symptoms, prevent

disease progression, improve exercise tolerance, improve health status, prevent and treat complications and exacerbations, and reduce mortality (***Rabe et al., 2007***).

1) Assessment of the disease

The goals of COPD assessment are to determine the severity of the disease, its impact on the patient's health status and the risk of future events (such as exacerbations, hospital admissions or death), in order to, eventually, guide therapy. To achieve these goals, COPD assessment must consider the following aspects of the disease separately (***GOLD, 2014***):

☐ Initial assessment

a) Current level of patient's symptoms

GOLD recommends the use of the Modified Medical Research Council (mMRC) questionnaire to assess symptoms in patients with COPD. The mMRC questionnaire assesses disability due to breathlessness, with mMRC grade ≥2 indicating a high level of symptoms; this predicts future mortality risk and relates well to other measures of health status (***Fletcher et al., 1959; Yawn, 2012***), Table (3).

Table (3). mMRC dyspnea scale (*GOLD, 2014*).

Grade 0	Breathless only with strenuous exercise.
Grade 1	Short of breath when hurrying on level ground or walking up a slight hill.
Grade 2	Walks slower on level ground than people of the same age because of breathlessness, or has to stop for breath when walking at own pace on the level.
Grade 3	Stops for breath after walking about 100 meters or after a few minutes on the level.
Grade 4	Too breathless to leave the house, or breathless when dressing or undressing.

b) Severity of the spirometric abnormality

COPD is graded according to severity, in terms of the FEV_1 measurement after a bronchodilator medication has been given to open up the airways. Patient's spirometric

classification is illustrated in Figure (1). GOLD categories 3 and 4 may be at greater risk of hospital admission and death (***Mannino and Buist, 2007; Yawn, 2012***).

In patients with $FEV_1/FVC < 0.70$:		
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

Figure (1). Classification of severity of airflow limitation in COPD (based on post-bronchodilator FEV_1) (***GOLD, 2014***).

c) Exacerbation risk:

An acute exacerbation of the disease is defined as an acute event characterized by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medication. The criteria of diagnosis is the presence of at least 2 major, or 1 major and 1 minor, symptom, on 2 or more consecutive days. Worsening dyspnea, increase in sputum volume, and increased sputum purulence are considered major symptoms, and increased cough or chest tightness, wheeze, sore throat, and coryza are considered minor symptoms (***Mokry et al., 2008; Caramori et al., 2009; Garvey and Ortiz, 2012; GOLD, 2014***).

There are two methods of assessing exacerbation risk. One is a population-based method using the GOLD spirometric classification, with GOLD 3 or GOLD 4 categories indicating high risk. The other is based on the individual patient's history of exacerbations, with two or more exacerbations in the preceding year indicating high risk. If there is a discrepancy between the risk category as assessed by spirometric classification and that derived from exacerbation history, the assessment pointing to the highest risk should be used (***Yawn, 2012; GOLD, 2014***).

d) Presence of comorbidities

COPD itself also has significant extra-pulmonary (systemic) effects including weight loss, nutritional abnormalities and skeletal muscle dysfunction, which can contribute to exercise intolerance and poor health status in patients with COPD. Cardiovascular disease is a major comorbidity in COPD and probably both the most frequent and most important disease coexisting with COPD. Other comorbidities that occur frequently in COPD patients include metabolic syndrome, osteoporosis, and depression. They are often under-diagnosed, and are associated with poor health status and prognosis (***GOLD, 2014***).

The existence of COPD may actually increase the risk for other diseases; this is particularly striking for COPD and lung cancer. It has been found to be the most frequent cause of death in patients with mild COPD. Whether this association is due to common risk factors (e.g., smoking), involvement of susceptibility genes, or impaired clearance of carcinogens is not clear (***Rabinovich and MacNee, 2011; Yawn, 2012***).

Comorbidities can occur in patients with mild, moderate or severe airflow limitation influence mortality and hospitalizations independently and deserve specific treatment. Therefore, comorbidities should be looked for routinely, and treated appropriately, in any patient with COPD. The guidelines for the diagnosis, assessment of severity, and management of individual comorbidities in patients with COPD are the same as for all other patients (***Mannino and Buist, 2007; GOLD, 2014***).

☐ Combined COPD Assessment

An understanding of the impact of COPD on an individual patient combines the symptomatic assessment with the patient's spirometric classification and/or risk of exacerbations. This approach to combined assessment is illustrated in Figure (2). Identifying patients in the "High Risk" groups combined with an assessment of potential comorbidities, reflects the complexity of COPD better than the unidimensional analysis of airflow limitation previously used for staging the disease and forms the basis of the guide to individualized management (***GOLD, 2014***). The groups can be summarized as follows (***Yawn, 2012; GOLD, 2014***):

- *Patient Group A – Low Risk, Less Symptoms*

Typically GOLD 1 or GOLD 2 (Mild or Moderate airflow limitation) and/or 0-1 exacerbation per year and no hospitalization for exacerbation; and CAT score < 10 or mMRC grade 0-1.

- *Patient Group B – Low Risk, More Symptoms*

Typically GOLD 1 or GOLD 2 (Mild or Moderate airflow limitation) and/or 0-1 exacerbation per year and no hospitalization for exacerbation; and CAT score ≥ 10 or mMRC grade ≥ 2.

- *Patient Group C – High Risk, Less Symptoms*

Typically GOLD 3 or GOLD 4 (Severe or Very Severe airflow limitation); and/or ≥ 2 exacerbations per year or ≥ 1 with hospitalization for exacerbation; and CAT score < 10 or mMRC grade 0-1.

- *Patient Group D – High Risk, More Symptoms*

Typically GOLD 3 or GOLD 4 (Severe or Very Severe airflow limitation) and/or ≥ 2 exacerbations per year or ≥ 1 with hospitalization for exacerbation; and CAT score ≥ 10 or and mMRC grade ≥ 2.

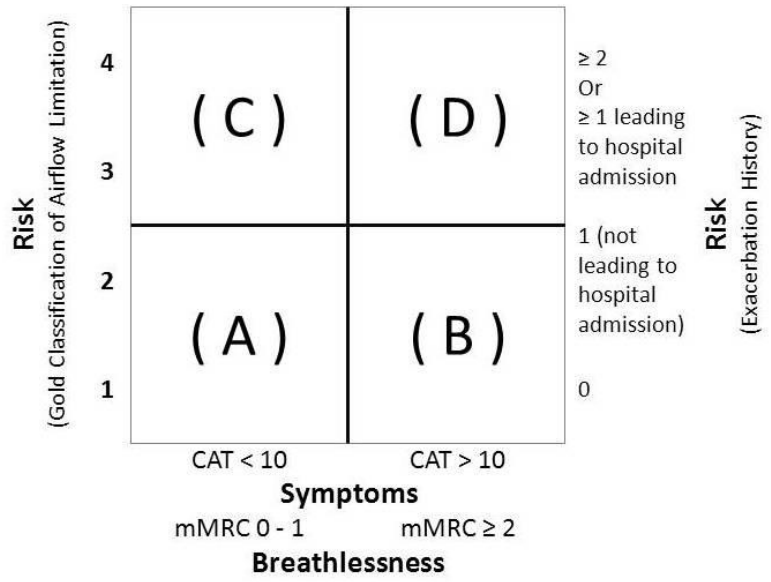


Figure (2). Assessment using Symptoms, Breathlessness, Spirometric Classification, and Risk of exacerbations (*GOLD, 2014*).

☐ Ongoing monitoring and assessment

a) Monitor disease progression and development of complications

COPD is usually a progressive disease. Lung function can be expected to worsen over time, even with the best available care. Symptoms and objective measures of airflow limitation should be monitored to determine when to modify therapy and to identify any complications that may develop. Follow-up visits should include a physical examination and discussion of symptoms, particularly any new or worsening symptoms. Spirometry should be performed if there is a substantial increase in symptoms or a complication (***Rabe et al., 2007; Casanova et al., 2011***).

b) Monitor pharmacotherapy and other medical treatment

To adjust therapy appropriately as the disease progresses, each follow-up visit should include a discussion of the current therapeutic regimen. Dosages of various medications, adherence to the regimen, inhaler technique, effectiveness of the current regime at controlling symptoms, and side effects of treatment should be monitored (***Rabe et al., 2007***).

c) Monitor exacerbation history

Frequency, severity, and likely causes of exacerbations should be evaluated. Severity can be estimated by the increased need for bronchodilator medication or glucocorticosteroids and by the need for antibiotic treatment. Hospitalizations should be documented, including the facility, duration of stay, and any use of critical care or intubation (***Rabe et al., 2007***).

d) Monitor comorbidities

Comorbidities may become harder to manage with COPD, either because they add to the total level of disability of COPD or because COPD therapy adversely affects the comorbid disorder. Until more integrated guidance about disease management for specific

comorbid problems becomes available, the focus should be on identification and management of these individual problems (***Rabe et al., 2007***).

2) Reduce risk factors

a) Smoking prevention and cessation

Smoking cessation is the single most effective—and cost-effective—way to reduce exposure to COPD risk factors. All smokers—including those who may be at risk for COPD as well as those who already have the disease—should be offered the most intensive smoking cessation intervention feasible. Pharmacotherapy for smoking cessation is recommended when counseling is not sufficient to help patients quit smoking. Nicotine replacement therapy in any form (nicotine gum, inhaler, nasal spray, transdermal patch, sublingual tablet, or lozenge) reliably increases long-term smoking abstinence rates. The antidepressants bupropion and nortriptyline have also been shown to increase long-term quit rates. Varenicline, a nicotinic acetylcholine receptor partial agonist that aids smoking cessation by relieving nicotine withdrawal symptoms and reducing the rewarding properties of nicotine, has been demonstrated to be safe and efficacious (***Rabe et al., 2007; GOLD, 2014***).

b) Occupational exposures

Many occupationally induced respiratory disorders can be reduced or controlled through a variety of strategies aimed at reducing the burden of inhaled particles and gases. The main emphasis should be on primary prevention, which is best achieved by the elimination or reduction of exposures to various substances in the workplace. Secondary prevention, achieved through surveillance and early case detection, is also of great importance (***Rabe et al., 2007; Calverley, 2010***).

c) Indoor and outdoor air pollution

Individuals experience diverse indoor and outdoor environments throughout the day, each of which has its own unique set of air contaminants and particulates that cause adverse

effects on lung function. Although outdoor and indoor air pollution are generally considered separately, the concept of total personal exposure may be more relevant for COPD. Reducing the risk from indoor and outdoor air pollution is feasible and requires a combination of public policy and protective steps taken by individual patients. Reduction of exposure to smoke from biomass fuel, particularly among women and children, is a crucial goal to reduce the prevalence of COPD worldwide. Although efficient nonpolluting cooking stoves have been developed, their adoption has been slow due to social customs and cost (***Rabe et al., 2007; GOLD, 2014***).

3) Management of stable COPD

The overall approach to managing stable COPD should be characterized by an increase in treatment, depending on the severity of the disease and the clinical status of the patient. Management of COPD is based on an individualized assessment of disease severity and response to various therapies. Treatment also depends on the patient's educational level and willingness to apply the recommended management (***Rabe et al., 2007; GOLD, 2014***).

a) Education

Patient education alone does not improve exercise performance or lung function, but it can play a role in improving skills, ability to cope with illness, and health status. Education also improves patient response to exacerbations. The topics that seem most appropriate for an education program include the following: smoking cessation; basic information about COPD and pathophysiology of the disease, general approach to therapy and specific aspects of medical treatment, self-management skills, strategies to help minimize dyspnea, advice about when to seek help, self-management and decision making during exacerbations, and advance directives and end-of-life issues (***Rabe et al., 2007; GOLD, 2014***).

b) Pharmacologic treatments

Pharmacologic therapy is used to prevent and control symptoms, reduce the frequency and severity of exacerbations, improve health status, and improve exercise tolerance. None of the medications used for COPD have been shown to modify the long-term decline in lung

function that is the hallmark of this disease. However, this should not preclude efforts to use medications to control symptoms (***Rabe et al., 2007; GOLD, 2014***).

Bronchodilators

Bronchodilator medications are central to the symptomatic management of COPD. They are given either on an as-needed basis for relief of persistent or worsening symptoms or on a regular basis to prevent or reduce symptoms. The side effects of bronchodilator therapy are pharmacologically predictable and dose dependent. Adverse effects are less likely, and resolve more rapidly after treatment withdrawal, with inhaled than with oral treatment (***Rabe et al., 2007; GOLD, 2014***).

Bronchodilator drugs commonly used in treating COPD include β_2-agonists, anticholinergics, and methylxanthines. The choice depends on the availability of the medications and the patient's response. All categories of bronchodilators have been shown to increase exercise capacity in COPD, without necessarily producing significant changes in FEV_1 (***Yawn, 2012; GOLD, 2014***), Table (4). Regular treatment with long-acting bronchodilators is more effective and convenient than treatment with short-acting brochodilators. Combining bronchodilators with different mechanisms and durations of action may increase the degree of bronchodilation for equivalent or lesser side effects (***Rabe et al., 2007; GOLD, 2014***).

Glucocorticoids

Regular treatment with inhaled glucocorticosteroids does not modify the long-term decline of FEV_1 in patients with COPD. However, it is appropriate for symptomatic patients with stages III and IV COPD and repeated exacerbations (three in the last 3 years). An inhaled glucocorticosteroid combined with a long-acting β_2-agonist is more effective than the individual components. Long-term treatment with oral glucocorticosteroids is not recommended in COPD. A side effect of long-term treatment with systemic glucocorticosteroids is steroid myopathy, which contributes to muscle weakness, decreased functionality, and respiratory failure in subjects with advanced COPD (***Woodhead et al., 2005; Yaw\n, 2012***).

Table (4). Therapy at each stage of COPD (*GOLD, 2014).*

Patient Group	Recommended First Choice	Alternative Choice	Other possible Treatment
A	Short-acting anticholinergic prn Or Short-acting β_2-agonist prn	Long-acting anticholinergic Or Long-acting β_2-agonist Or Short-acting β_2-agonist and short-acting anticholinergic	Theophylline
B	Long-acting anticholinergic Or Long-acting β_2-agonist	Long-acting anticholinergic and long-acting β_2-agonist	Short-acting β_2-agonist And/or Short-acting anticholinergic Theophylline
C	Inhaled corticosteroids + Long-acting β_2-agonist Or Long-acting anticholinergic	Long-acting anticholinergic and long-acting β_2-agonist Or Long-acting anticholinergic and phosphodiesterase-4 inhibitor Or Long-acting β_2-agonist and phosphodiesterase-4 inhibitor	Short-acting β_2-agonist And/or Short-acting anticholinergic Theophylline
D	Inhaled corticosteroids + Long-acting β_2-agonist And/or Long-acting anticholinergic	Inhaled corticosteroids + long-acting β_2-agonist and long-acting anticholinergic Or Inhaled corticosteroids + long-acting β_2-agonist and phosphodiesterase-4 inhibitor Or Long-acting anticholinergic and long-acting β_2-agonist Or Long-acting anticholinergic and phosphodiesterase-4 inhibitor	Carbocysteine Short-acting β_2-agonist And/or Short-acting anticholinergic Theophylline

Other pharmacologic treatments

Vaccines. Influenza vaccines can reduce serious illness and death in patients with COPD by approximately 50%. Vaccines containing killed or live, inactivated viruses are recommended because they are more effective in elderly patients with COPD. They should be given once each year. Pneumococcal polysaccharide vaccine is also recommended for

patients with COPD who are 65 years and older. In addition, this vaccine has been shown to reduce the incidence of community-acquired pneumonia in patients with COPD who are younger than 65 years with an $FEV_1 < 40\%$ predicted (***Alfageme et al., 2006***).

α_1 -antitrypsin augmentation therapy. Young patients with severe hereditary a1-antitrypsin deficiency and established emphysema may be candidates for α_1-antitrypsin augmentation therapy (***Rabe et al., 2007***).

Antibiotics. Prophylactic, continuous use of antibiotics, other than for treating infectious exacerbations of COPD and other bacterial infections, has been shown to have no effect on the frequency of exacerbations in COPD (***Bekkers et al., 2010***).

Mucolytic agents. The regular use of mucolytics in COPD has been evaluated in a number of long-term studies with controversial results. Although a few patients with viscous sputum may benefit from mucolytics, the overall benefits seem to be very small, and the widespread use of these agents are not be recommended at present (***Rabe et al., 2007; Yawn, 2012***).

Antioxidant agents. Antioxidants, in particular N-acetylcysteine, have been reported in small studies to reduce the frequency of exacerbations. However, a large randomized controlled trial found no effect of N-acetylcysteine on the frequency of exacerbations, except in patients not treated with inhaled glucocorticosteroids (***Rabe et al., 2007***).

Immunoregulators. Studies using an immunoregulator in COPD show a decrease in the severity and frequency of exacerbations (***GOLD, 2014***).

Antitussives. Cough has a significant protective role. But the regular use of antitussives is not recommended in stable COPD (***GOLD, 2014***).

Vasodilators. In patients with COPD, inhaled nitric oxide can worsen gas exchange because of altered hypoxic regulation of ventilation–perfusion balance. Therefore, based on the available evidence, nitric oxide is not indicated in stable COPD (***Rabe et al., 2007***).

Narcotics (morphine). Oral and parenteral opioids are effective for treating dyspnea in patients with advanced COPD disease. However, some clinical studies suggest that

morphine used to control dyspnea may have serious adverse effects and its benefits may be limited to a few sensitive subjects (***Rabe et al., 2007***).

Others. Nedocromil, leukotriene modifiers, and alternative healing methods (e.g., herbal medicine, acupunture, and homeopathy) have not been adequately tested in patients with COPD and thus cannot be recommended at this time (***GOLD, 2014***).

c) Non-pharmacologic treatments

Rehabilitation

The principal goals of pulmonary rehabilitation are to reduce symptoms, improve quality of life, and increase physical and emotional participation in everyday activities. To accomplish these goals, pulmonary rehabilitation covers a range of non-pulmonary problems that may not be adequately addressed by medical therapy for COPD. Such problems, which especially affect patients with stages II through IV COPD, include exercise deconditioning, relative social isolation, altered mood states, muscle wasting, and weight loss. The components of pulmonary rehabilitation include exercise training, nutrition counseling, and education (***Rabe et al., 2007; Burtin et al., 2011; Rabbat, 2011***).

Oxygen therapy

The long-term administration of oxygen (>15 h/d) to patients with chronic respiratory failure has been shown to increase survival and improve hemodynamics, hematologic characteristics, exercise capacity, lung mechanics, and mental state. The primary goal of oxygen therapy is to increase the baseline PaO_2 to at least 8.0 kPa (60 mm Hg) at sea level and rest, and/or produce a Sa O_2 of at least 90%, which will preserve vital organ function by ensuring adequate delivery of oxygen (***Rabe et al., 2007***).

Ventilator support

Although long-term noninvasive positive-pressure ventilation (NIPPV) cannot be recommended for the routine treatment of patients with chronic respiratory failure due to COPD, the combination of NIPPV with long-term oxygen therapy may be of some use in a

selected subset of patients, particularly in those with pronounced daytime hypercapnia (***Rabe et al., 2007***).

Surgical treatments

Bullectomy is indicated when the bulla occupies more than 50% of the hemithorax and produces definite displacement of the adjacent lung. Whereas, lung volume reduction surgery is indicated when FEV_1 > 35% of predicted, $PaCO_2$ <45 mmHg, predominant upper lobe, emphysema on CT scan and a residual volume of <200 % of predicted. In appropriately selected patients with very advanced COPD, lung transplantation has been shown to improve quality of life and functional capacity. Criteria for referral for lung transplantation include: FEV_1<35% predicted, PaO_2< 55-60 mmHg, $PaCO_2$>50 mmHg, and secondary pulmonary hypertension (***Naunheim et al., 2006***).

4) Management of Exacerbations

Exacerbations are categorized in terms of either clinical presentation (number of symptoms) and/or heath care resources utilization. The impact of exacerbations is significant and a patient's symptoms and lung function may both take several weeks to recover to the baseline values (***Garvey and Ortiz, 2012***). The most common causes of an exacerbation are infection of the tracheobronchial tree and air pollution, but the cause of approximately one-third of severe exacerbations cannot be identified. Conditions that may mimic an exacerbation include pneumonia, congestive heart failure, pneumothorax, pleural effusion, pulmonary embolism and arrhythmia (***Rubio et al., 2010; Yawn, 2012***).

Diagnosis and assessment of severity

Increased breathlessness, the main symptom of an exacerbation, is often accompanied by wheezing and chest tightness, increased cough and sputum, change of the color and / or tenacity of sputum and fever. Exacerbation may also be accompanied by a number of nonspecific complaints such as malaise, insomnia sleepiness, fatigue, depression and confusion (***Rabe et al., 2007***; ***Garvey and Ortiz, 2012***).

Assessment of the severity of an exacerbation is based on the patient's medical history before the exacerbation, symptoms, physical examination, lung function tests, arterial blood gas measurements and other laboratory tests. Signs of a severe exacerbation are use of accessory respiratory muscle, paradoxical chest wall movements, worsening or new onset central cyanosis, development of peripheral edema, hemodynamic instability, signs of right heart failure, and reduced alertness. Pulmonary function test is difficult to be performed properly, and are not accurate during an acute exacerbation; therefore, their routine use is not recommended (***Hoogendoorn et al., 2011; Garvey and Ortiz, 2012***).

Pulse oximetry can be used to evaluate a patient's oxygen saturation and need for supplemental oxygen therapy. For patients that require hospitalization, measurement of arterial blood gases is important to assess the severity of an exacerbation. A PaO_2 <8.0 kPa (60 mm Hg) and /or SaO_2 < 90% with or without $PaCO_2$ > 6.7 kPa (50 mm Hg) when breathing room air indicate respiratory failure. Whereas, a PaO_2 < 6.7 kPa (50 mm Hg), $PaCO_2$ > 9.3 kPa (70 mm Hg), and PH < 7.30 point towards a life-threatening episode that needs critical management (***GOLD, 2014***).

Chest radiographs (posterior/anterior plus lateral) are useful in identifying alternative diagnoses that can mimic the symptoms of an exacerbation. An ECG aids in the diagnosis of right heart hypertrophy, arrhythmias, and ischemic episodes. Pulmonary embolism can be very difficult to distinguish from an exacerbation, especially in advanced COPD, spiral CT scanning and angiography, and perhaps specific D-dimer assays, are the best tools presently available for the diagnosis of pulmonary embolism in patients with COPD but ventilation-perfusion scanning is of no value (***Rabe et al., 2007; GOLD, 2014***).

The whole blood count may identify polycythemia (hematocrit > 55%) or bleeding. White blood cell counts are usually not very informative. The presence of purulent sputum during an exacerbation of symptoms is sufficient indication for starting empirical antibiotic treatment. Streptococcus pneumoniae, hemophilis influenzae and moraxella catarrhalis are the most common bacterial pathogens involved in COPD exacerbations. If an infectious exacerbation does not respond to the initial antibiotic treatment, a sputum culture and an

antibiogram should be performed. Biochemieal tests can reveal whether the cause of the exacerbation is an electrolyte disturbance (***Rabbat et al., 2010; GOLD, 2014***).

Home management

The goals of treatment for COPD exacerbations are to minimize the impact of the current exacerbation and prevent the development of subsequent exacerbations. Depending on the severity of an exacerbation and/or the severity of the underlying disease, an exacerbation can be managed in an outpatient or inpatient setting (***Garvey and Ortiz, 2012***). The three classes of medications most commonly used for exacerbations of COPD are bronchodilators, corticosteroids, and antibiotics (***Hernandez et al., 2003***).

Table (5). Indications for Hospital Assessment or Admission for Exacerbation of COPD (*GOLD, 2014*).

- Marked increase in intensity of symptoms, such as sudden development of resting dyspnea.
- Severe underlying COPD.
- Onset of new physical signs (e.g., cyanosis, peripheral edema).
- Failure of exacerbation to respond to initial medical management.
- Significant comorbidities.
- Frequent exacerbations
- Older age.
- Insufficient home support.

Home management of COPD exacerbations involves increasing the dose and/or frequency of existing short-acting bronchodilator therapy, preferably with a β_2-agonist. If not already used, an anticholinergic can be added until the symptoms improve. Antibiotics are usually needed during COPD exacerbations (***Rodriguez-Roisin, 2006***). Systemic glucocorticosteroids shorten recovery time, improve lung function (FEV_1) and hypoxemia (PaO_2), and may reduce the risk of early relapse, treatment failure, and length of hospital

stay. They should be considered in addition to bronchodilators if the patient's baseline FEV_1 is less than 50% predicted (***GOLD, 2014***).

Hospital management

A range of criteria to consider for hospital assessment/admission for exacerbations of COPD, Table (5).The first actions when a patient reaches the emergency department are to provide supplemental oxygen therapy and to determine whether the exacerbation is life threatening. Guidelines of management of severe but not life-threatening exacerbation of COPD in the emergency department or hospital are listed in Table (6). Some patients need immediate admission to an intensive care unit (ICU), Table (7) ***(GOLD, 2014)***.

Table (6). Management of severe but not life-threatening exacerbations of COPD in the emergency department or the hospital (*GOLD, 2014*).

- Assess severity of symptoms, blood gases, chest radiograph
- Administer supplemental oxygen therapy and obtain serial arterial blood gas measurement
- Bronchodilators:
  - Increase doses and/or frequency of short-acting bronchodilators
  - Combine short-acting beta2-agonists and anticholinergics
  - Use spacers or air-driven nebulizers
- Add oral or intravenous corticosteroids
- Consider antibiotics (oral or occasionally intravenous) when signs of bacterial infection
- Consider noninvasive mechanical ventilation
- At all times:
  - Monitor fluid balance and nutrition
  - Consider subcutaneous heparin or low molecular weight heparin
  - Identify and treat associated conditions (e.g., heart failure, arrhythmias)
  - Closely monitor condition of the patient

a) Controlled oxygen therapy

Oxygen therapy is the cornerstone of hospital treatment of COPD exacerbations. Adequate levels of oxygenation (PaO_2 > 8.0 kPa, 60 mm Hg, or SaO_2 > 90%) are easy to achieve in uncomplicated exacerbations, but CO_2 retention can occur insidiously with little

change in symptoms. Once oxygen is started, arterial blood gases should be checked 30 minutes later to ensure satisfactory oxygenation without CO_2 retention or acidosis ***(Celli and MacNee, 2004; Rabe et al., 2007; Yawn, 2012)***.

b) Bronchodilator therapy

Short-acting inhaled β_2-agonists are usually the preferred bronchodilators for treatment of exacerbation of COPD. If a prompt response to these drugs does not occur, the addition of an anthicholinergic is recommended. In more severe exacerbations, addition of an oral or intravenous methylxanthine to the treatment can be considered (***Garvey and Ortiz, 2012).***

Table (7). Indications for ICU Admission of Patients with Exacerbation of COPD (*GOLD, 2014*).

- Severe dyspnea that responds inadequately to initial emergency therapy.
- Change in mental status (confusion, lethargy, coma).
- Persistent or worsening hypoxemia (PaO2 < 5.3 kPa , 40 mm Hg), and or severe/ worsening hypercapnia (PaCO2 > 8.0 kPa, 60 mm Hg) and/or severe / worsening respiratory acidosis (pH < 7.25) despite supplemental oxygen and noninvasive ventilation
- Need for invasive mechanical ventilation
- Hemodynamic instability, need for vasopressors

c) Glucocorticosteroids

Oral or intravenous glucocorticosteroids are recommended as an addition to bronchodilator therapy (plus eventually antibiotics and oxygen therapy) in the hospital management of exacerbation of COPD. Thirty to 40 mg of oral prednisolone daily for 10 to 14 days is a reasonable compromise between efficacy and safety (***Garvey and Ortiz, 2012; GOLD, 2014***).

d) Antibiotics

Antibiotics should be given to patients with exacerbations of COPD with the following three cardinal symptoms: increased dyspnea, increased sputum volume, and increased

sputum purulence, or to patients with a severe exacerbation of COPD that requires mechanical ventilation (***Celli and MacNee, 2004; GOLD, 2014***).

e) Ventilatory support

The primary objectives of mechanical support in patients with exacerbation are to decrease mortality and morbidity and to relieve symptoms. Ventilatory support includes both noninvasive mechanical ventilation using either negative or positive pressure devices, and invasive (conventional) mechanical ventilation by oro/naso-tracheal tube or tracheostomy. The indications for initiating non-invasive mechanical ventilation (NIMV) and invasive mechanical ventilation during exacerbations of COPD are shown in Table (8) and (9) (***Rabe et al., 2007; Gold, 2014***).

Table (8). Indications for non-invasive mechanical ventilation (*GOLD, 2014*).

- At least one of the following:
  - Respiratory acidosis (arterial pH ≤ 7.35 and/or PaCO2 ≥ 6.0 kPa, 45 mm Hg)
  - Severe dyspnea with clinical signs suggestive of respiratory muscle fatigue, increased work of breathing, or both, such as use of respiratory accessory muscles, paradoxical motion of the abdomen, or retraction of the intercostal spaces

Table (9). Indications for invasive mechanical ventilation (*GOLD, 2014*).

- Unable to tolerate NIV or NIV failure
- Respiratory or cardiac arrest
- Respiratory pauses with loss of consciousness or gasping for air
- Diminished consciousness, psychomotor agitation inadequately controlled by sedation
- Massive aspiration
- Persistent inability to remove respiratory secretions
- Heart rate < 50/ min with loss of alertness
- Severe hemodynamic instability without response to fluids and vasoactive drugs
- Severe ventricular arrhythmias
- Life-threatening hypoxemia in patients unable to tolerate NIV
- Other complications (metabolic abnormalities, sepsis, pneumonia, pulmonary embolism , barotrauma, massive pleural effusion)

Hospital discharge and follow-up

Close follow-up assessment 4 to 6 weeks after discharge from the hospital should be conducted, Table (10). Thereafter, follow-up is the same as for patients with stable COPD, including supervising smoking cessation, monitoring the effectiveness of each drug treatment, and monitoring changes in spirometric parameters. Home visits by a community nurse may permit earlier discharge of patients hospitalized with an exacerbation of COPD, without increasing readmission rates (***Rabe et al., 2007***; ***Garvey and Ortiz, 2012***). In patients who are hypoxemic during a COPD exacerbation, arterial blood gases and/or pulse oximetry should be evaluated before hospital discharge and in the following 3 months. If the patient remains hypoxemic, long-term supplemental oxygen therapy may be required (***Hoogendoorn et al., 2011***).

Table (10). Discharge Criteria for Patients with Exacerbation of COPD (*GOLD, 2014*).

- Able to use long acting bronchodilators, either β_2-agonist and/or anticholinergics with or without inhaled corticosteroids
- Inhaled β_2-agonist therapy is required no more frequently than every 4 hrs.
- Patient, if previously ambulatory, is able to walk across room.
- Patient is able to eat and sleep without frequent awakening by dyspnea.
- Patient has been clinically stable for 12-24 hrs.
- Arterial blood gases have been stable for 12-24 hrs.
- Patient (or home caregiver) fully understands correct use of medications.
- Follow-up and home care arrangements have been completed (e.g., visiting nurse, oxygen delivery& meal provisions).
- Patient, family and physician are confident that the patient can manage at home successfully.

Opportunities for prevention of future exacerbations should be reviewed before discharge, with particular attention to smoking cessation, current vaccination (influenza, pneumococcal vaccines), knowledge of current therapy including inhaler technique, and how to recognize symptoms of exacerbations. Pharmacotherapy known to reduce the

number of exacerbations and hospitalizations and delay the time of first/ next hospitalization, such as long-acting inhaled bronchodilators, inhaled glucocorticosteroids, and combination inhalers, should be specifically considered (***Garvey and Ortiz, 2012; Yawn, 2012***).

Pharmacogenetics of COPD

Inhaled bronchodilators and inhaled corticosteroids (ICS) are the most commonly used medications in the management of patients with stable COPD. However, many COPD patients remain symptomatic despite medical therapy (***Hersh, 2010***). The heterogeneity of COPD and COPD phenotypes is becoming increasingly recognized (***Reilly, 2008***). The classic dichotomy of COPD subtypes includes emphysema, characterized by destruction of the lung tissue, and chronic bronchitis, characterized by inflammation of the large airways. Small airway disease is also an important cause of airflow obstruction (***Hogg et al., 2004***). However, these major COPD phenotypes may co-exist in the same patient, and there are likely to be important sub-phenotypes based on combinations of these and other processes. If these COPD phenotypes are genetically determined, then the relevant genes will be important markers of response to therapy. β_2-adrenergic receptor is one of the candidate genes analyzed in COPD pharmacogenetic studies ***(*Hersh, *2010***).

β_2-adrenergic receptor

The β_2-adrenergic receptor (β_2-AR), also known as *ADRB2*, is a membrane-bound G-protein coupled receptor that is widely distributed in the body, and mediates some of the physiological actions of catecholamines (noradrenaline and adrenaline) in a variety of tissues (***Daghestani et al., 2012***).

Distribution of β_2-adrenergic receptor

In human lung, β_2-adrenoceptors are located throughout the lungs, from the trachea to the alveoli. They are expressed in many cell types, including bronchial smooth muscle cells, airway epithelial cells, alveolar walls, vascular endothelium, mononuclear cells, neutrophils, eosinophils, alveolar macrophages, and presynaptic cholinergic nerve terminals. Strikingly, the receptor expression increases with each airway generation, with the greatest total amounts in the distal airways and alveoli. They are also expressed in cardiac myocytes and

vascular smooth muscle cells but with receptor density lower than pulmonary β_2-receptors (***Mutlu and Factor, 2008; Augusto et al., 2010***).

Function of β_2-adrenergic receptor

The β_2-AR mediates various physiologic responses of the airways, including bronchodilation, improvement of mucociliary clearance, and various anti-inflammatory actions as mast cell stabilization, and reduction of protein extravasation in the airways. In cardiac tissues, β_2-AR causes an increase in the rate and force of heart contractions (***Yang et al., 2007; Augusto et al., 2010***).

Structure of β_2-adrenergic receptor

The β_2-AR is a single polypeptide chain of 413 amino acid residues with a molecular weight of 46.5 KD (Kilodaltons). It is composed of an extracellular amino terminus, seven transmembrane-spanning domains, 3 intracellular and 3 extracellular loops, and an intracellular carboxyl terminus (***Rasmussen et al., 2011***), Figure (3).

After formation of the polypeptide chain, the receptor undergoes three types of post-translational modification that can alter protein structure and molecular weight: glycosylation, palmitoylation, and disulfide bond formation, Figure (4). N-linked glycosylation is the addition of N-linked sugars to the protein at amino acids 6, 15, and 187. These glycosylation is responsible for membrane insertion and agonist-induced receptor trafficking (***Rasmussen et al., 2007 and 2011***).

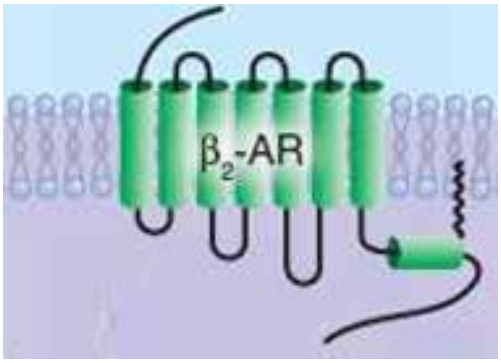


Figure (3). The human beta$_2$ adrenergic receptor structure (***Audet and Bouvier, 2008***).

Palmitoylation occurs at the cysteine residue of amino acid 341, and is responsible for reversible anchoring of the carboxy-terminus to the membrane. Palmitoylation has been found to stimulate agonist-induced adenylyl cyclase (AC) activity and affects ADRB2 phosphorylation and desensitization. The region between the seventh

transmembrane-spanning domain and the palmitoylated cysteine is sometimes denoted as the fourth intracellular loop (***Johnson, 2006***).

Disulfide bonds formation has been found to occur at cysteine residues 106, 184, 190, and 191. These bonds function to stabilize the ADRB2 ligand-binding pockets (***Rasmussen et al., 2011***). β_2-AR also contains phosphorylation sites in the third intracellular loop and proximal cytoplasmic tail. Phosphorylation of these sites triggers the agonist-promoted desensitization, internalization, and degradation of the receptor (***Sato et al., 2011***).

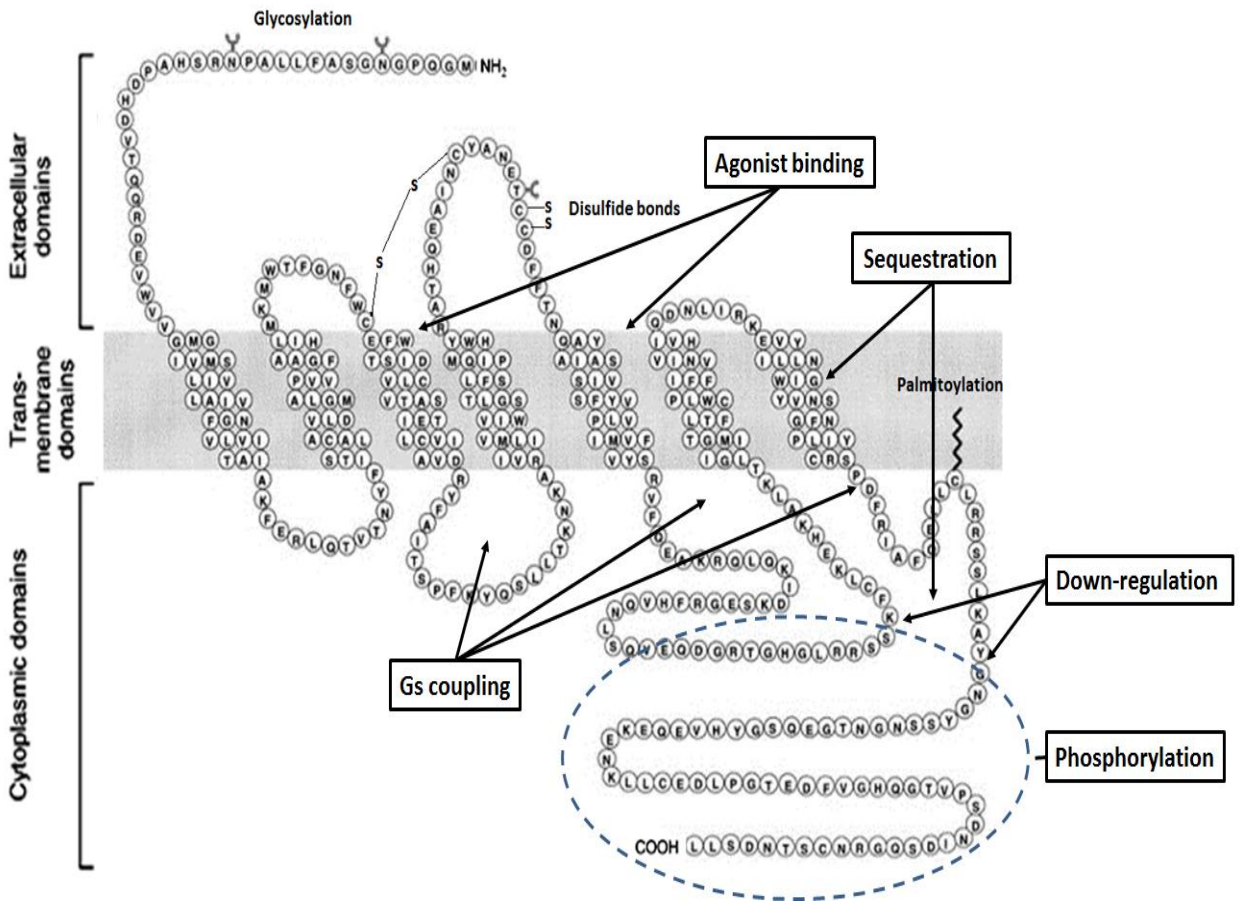


Figure (4). The primary amino acid sequence of beta$_2$ adrenergic receptor. *Single letter* amino acid nomenclature is used. Agonist binding to ADRB2 occurs in the extracellular pockets between the third to fourth and fifth to sixth transmembrane domains (modified from ***Liggett., 2002; Rasmussen et al., 2007***).

Biological activity of β_2-adrenergic receptor

Ligand binding and receptor activation

β_2-adrenoceptors exist in the plasma membrane in an equilibrium between two structural conformations; inactive and active forms. Like many G-protein coupled receptors, β_2-AR spontaneously oscillates between inactive and active conformations. In the absence of agonist, the basal equilibrium favors the inactive form. Engagement of the β_2-AR by a β_2-agonist produces a conformational change shifting the equilibrium toward the active form and causing its association with the stimulatory heterotrimeric G-protein (Gs), which contains three subunits G_α, G_β and G_γ (***Mutlu and Factor, 2008; Sato et al., 2011***).

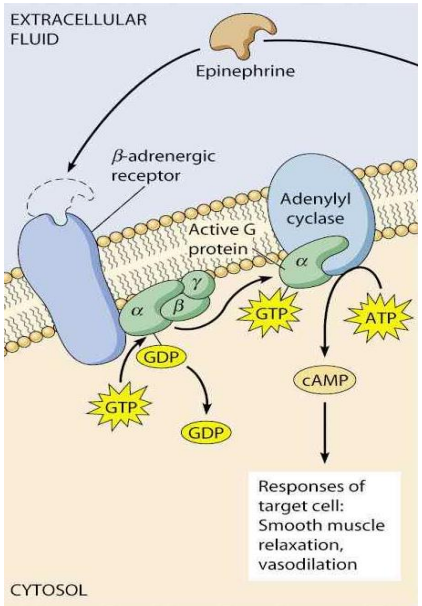


Figure (5). The β_2-AR activation (***silverman et al., 2001***).

Coupling of Gs to the activated receptor causes G_α to exchange its GDP (guanosine diphosphate) for GTP (guanosine triphosphate). GTP catalyzes dissociation of the G-protein heterotrimer into activated G_α and $G_{\beta\gamma}$ subunits, inducing the receptor to switch back to its low-energy inactivated form (***Augusto et al., 2010; Staus et al., 2014***), Figure (5).

Intracellular signaling pathways

Activation of β_2-AR results in a variety of distinct signaling events. In the traditional pathway, dissociated G_α couples with and activates adenylyl cyclase, which catalyzes the conversion of ATP into cAMP (cyclic adenosine monophosphate). This cAMP, in turn, activates protein kinase A (PKA), which induces phosphorylation of various downstream proteins involved in the control of muscle tone; including phospholipase C, cell surface potassium channels, myosin light-chain kinase, and β_2-AR itself. cAMP also results in inhibition of calcium ion (Ca^{2+}) release from intracellular stores, and sequestration of

intracellular Ca^{2+}. The net effect is phosphorylation of contractile proteins and a decrease in intracellular Ca^{2+}, leading to bronchial relaxation and airway dilatation (***Silverman et al., 2001; Johnson, 2006; Sato et al., 2011***), Figure (6a).

Although most of the actions of the β_2-receptor appear to be mediated through Gs proteins and the cAMP-dependent PKA system, studies revealed that some of the relaxant response to β_2-agonists might be mediated through cAMP-independent mechanisms, involving direct interaction of Gs with potassium channels, which are present in the airway smooth muscle cell membrane (***Johnson, 2006; Chung et al., 2011; Daghestani et al., 2012***).

Phosphorylated β_2-receptors can bind to $\beta\gamma$-subunits of the inhibitory G (Gi) protein, resulting in stimulation of mitogen-activated protein kinase (MAPK) pathway. Activation of this pathway might serve to uncouple the β_2-receptor from Gs and thus represent means of terminating the β_2-agonist/receptor signal and response. In addition, β_2-receptor–activated MAPK phosphorylates the glucocorticoid receptor (GR) at its N-terminal region, rendering the receptor more sensitive to steroid-dependent activation. β_2-AR activation also increases the translocation of the GR from the cell cytosol to the nucleus, a fundamental step in the anti-inflammatory activity of corticosteroids. These mechanisms provide a molecular basis for synergy between the β2-receptor and the GR (***Chung et al., 2011***).

β_2-receptors are also important in lung fluid clearance by two mechanisms. Stimulation of the ADRB2 on types I and II alveolar cells results in an increase in the activity and/or number of epithelial sodium channels (ENaC) on the apical membrane of these cells. This increase in ENaC activity augments sodium and, therefore, water flux from the alveolar airspace into the cell. Fluid transport is completed on the basolateral portion of these cells via the Na^+/K^+ transporter. In addition, the ADRB2 have been localized to the pulmonary lymphatics, and stimulation of these receptors on the lymphatics can result in lung fluid (***Synder et al., 2008***).

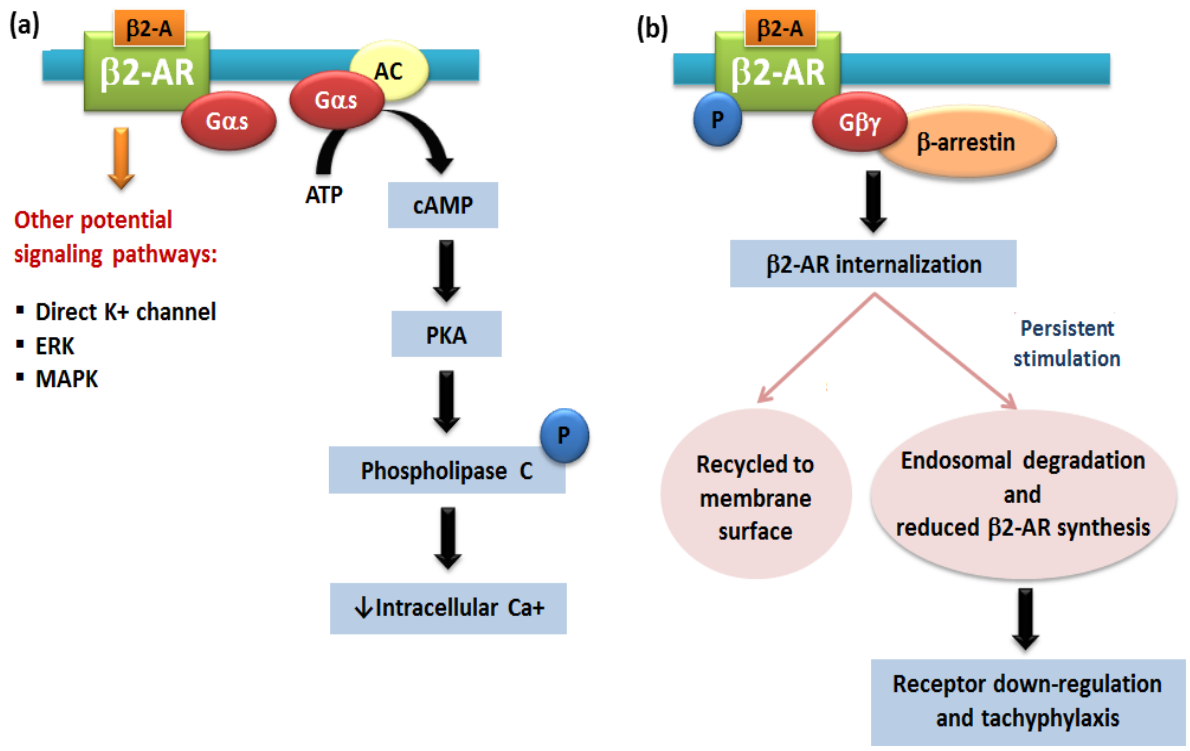


Figure (6). Schematic diagram of conventional β_2-adrenergic receptor signaling pathway and receptor trafficking upon activation by β_2-adrenergic receptor agonists (***Chung et al., 2011***).
(a) Binding of β_2-AR agonists (β_2-A) to β_2-AR results in binding to the α-subunit of stimulatory G protein ($G_{\alpha s}$), which leads to activation of adenylyl cyclase (AC) and a consequent increase in cAMP. cAMP activates protein kinase A (PKA), which induces phosphorylation (P) of various downstream proteins including phospholipase C and β_2-AR itself. Phopholipase C, together with other potential various mechanisms, e.g. direct K channels, reduces intracellular calcium (Ca+) thereby causing bronchodilatation. (b) Phosphorylated β_2-AR, however, is uncoupled from $G_{\alpha s}$ and binds to the β and γ subunits of inhibitory G protein ($G_{\beta\gamma}$). This terminates the receptor activation signal and stimulates binding to β-arrestin, causing receptor internalization. Receptors are recycled back to the membrane upon prompt removal of the agonist. However, with persistent receptor activation, receptors are down-regulated through degradation of internalized receptors and reduced synthesis of new receptors.

Regulation of β_2-AR expression

Continuous activation of β_2-AR initiates negative feedback that lead to a rapid attenuation of the biological response, a process known as receptor desensitization. This process operates as a safety device to prevent overstimulation of receptors in the face of excessive β_2-agonist exposure (***Lynch and Ryall, 2008; Chung et al., 2011***). The mechanisms by which desensitization can occur consist of 3 main processes: (1) phosphorylation leading to uncoupling from G_s and AC, (2) internalization of uncoupled receptors where the receptor can be degraded or recycled, and (3) down-regulation of receptor number by alterations in receptor gene expression (***Stephen, 1999***). The extent of desensitization depends on the degree and duration of the β_2-receptor/β_2-agonist response (***Johnson, 2006***), Figure (6b).

Phosphorylation of β_2-AR occurs rapidly after agonist exposure and is mediated partially by cAMP–dependent PKA and by β-AR kinase (βARK; also termed G protein–coupled receptor kinases "GRK2"). This terminates the receptor activation signal and stimulates binding to β-arrestin protein, that serves to decrease coupling of the receptor to G_s and recruits other proteins as phosphodiesterase (PDE) to metabolize locally generated cAMP, thereby limiting receptor function (***Johnson, 2006***; ***Lynch and Ryall, 2008; Augusto et al., 2010***).

After more prolonged exposure to agonist (seconds to minutes), a proportion of β_2-AR internalizes into subcellular compartments through clathrin-mediated endocytosis, a process termed sequestration. These sequestered receptors are not available for signal transduction and thus can act as a mechanism of desensitization. They can be recycled back to the cell surface (resensitization) or transferred to lysosomes for degradation. Internalization takes longer to reverse than uncoupling, but full reversal normally occurs within hours of removal of the agonist (***Stephen, 1999; Johnson, 2006; Chung et al., 2011; Sato et al., 2011***).

However, with persistent receptor stimulation (hours to days), receptors are down-regulated through increased degradation, as well as decreased synthesis of new receptors. Receptor desensitization and down-regulation manifest clinically as tachyphylaxis to β_2-AR-agonist therapy (***Chung et al., 2011***).

β_2-AR / agonist interactions

The molecular structure of a β_2-agonist determines the manner in which it interacts with the β_2-adrenoceptor. Short-acting β_2-agonists, such as albuterol, which are hydrophilic in nature, access the active site of the β_2-adrenoceptor directly from the extracellular aqueous compartment (***Johnson et al., 2000***). There is therefore a rapid onset of action. However, these drugs rapidly re-equilibrate, their residency time at the receptor active site is limited, and the resulting duration of action is short (4-6 hours) (***Johnson et al., 2006***).

The long-acting β_2-agonists have different mechanisms of action. Formoterol is moderately lipophilic, and it is taken up into the cell membrane in the form of a depot, from where it progressively leaches out to interact with the active site of the β_2-receptor (***Johnson et al., 200***0). The size of the depot is determined by the concentration or dose of formoterol applied. The onset of action of formoterol is somewhat delayed compared with that of albuterol, and the duration of activity is longer and concentration dependent (***Ringdahl et al., 1995; Johnson et al., 2006***).

Salmeterol, on the other hand, is more than 10,000 times more lipophilic than albuterol. The molecule partitions rapidly (<1 minute) into the cell membrane and then diffuses laterally to approach the activity site of the β_2-adrenoceptor through the membrane, with this process appearing to be slow (>30 minutes). The onset of action of salmeterol in airway smooth muscle is therefore slower than that of other β_2-agonists, such as albuterol and formoterol. The mechanism of action of salmeterol involves the interaction of the side chain of the molecule with an auxiliary binding site (exosite), a domain of highly hydrophobic amino acids within the fourth domain of the β_2-adrenoceptor (***Johnson et al., 1993; Ind et al., 1997; Szczuka et al., 2009; Plazinska et al., 2013***).

The affinity of a ligand is a measure of the avidity of its binding to its receptor. Few β_2-agonists have been shown to have much higher affinity than isoproterenol, and indeed, albuterol has a relatively low affinity for β_2-adrenoceptors. In contrast, salmeterol and formoterol have 3- to 4-fold higher affinities for the β_2-adrenoceptor than albuterol. β_2-agonist potency, however, is a function not only of receptor affinity but also of efficacy. A

full agonist will have a high efficacy, whereas a pure antagonist will have low or zero efficacy (***Johnson et al., 2006***).

The majority of β_2-adrenoceptor agonists have an intermediate efficacy, and if receptor numbers permit, they will behave as full agonists, but if receptor density is too low or receptor coupling is inadequate, the β_2-agonist might behave in a partial manner; that is, it will be incapable of achieving the same maximum effect as an agonist of higher efficacy, and it might even behave as an antagonist. Examples of compounds of high efficacy (full agonists) are isoproterenol, fenoterol, and formoterol, whereas albuterol and terbutaline are of moderate efficacy (partial agonists). Salmeterol has an efficacy at β_2-adrenoceptors in airway smooth muscle lower than that of albuterol. Low efficacy in a β_2-adrenoceptor agonist does not, however, compromise clinical performance as a bronchodilator drug but might limit its effectiveness in restoring airway caliber under conditions of acute severe bronchospasm (***Johnson et al., 2006; Szczuka et al., 2009***).

Pharmacologically, a partial agonist will be required to occupy a higher proportion of receptors than a full agonist to exert its maximum effect. Ex vivo studies in asthmatic patients have shown salmeterol at clinical doses to occupy only 4% of the available pulmonary β_2-receptors. This represents a receptor reserve of 96% and is reflected in a normal bronchodilator response to albuterol, when used as rescue therapy, in patients chronically treated with salmeterol (***Johnson et al., 2000***).

Genetics of β_2-adrenergic receptor

β_2-adrenergic receptor gene

The gene encoding the β_2-adrenergic receptor, *ADRB2* gene (MIM# 109690), is located on the long arm of chromosome 5 at region 3, band 1 to 2 (5q31–q32). *ADRB2* gene lacks introns and consists of a single exon. Its full-length mRNA spans 2033 nucleotides (nt), including the 5'-and 3'-untranslated regions (UTR) (NCBI NM_000024). The protein-coding region spans 1239 nt within the full-length mRNA, these specific nucleotides encode

the 413 amino acid residues forming the receptor (***Augusto et al., 2010; Chung et al., 2011***), Figure (7).

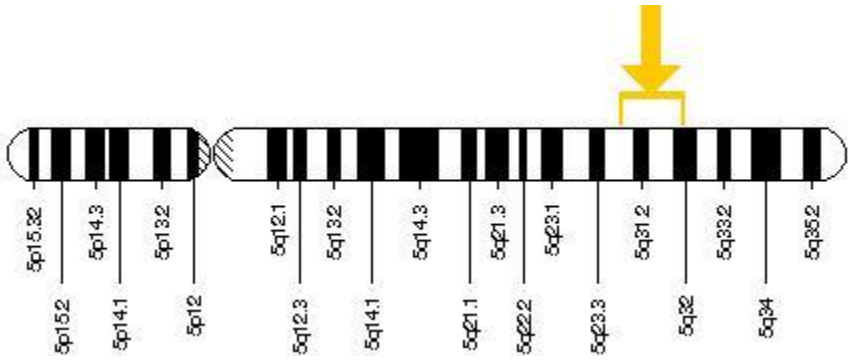


Figure (7). Location of *ADRB2* gene on chromosome 5 (http://ghr.nlm.nih.gov**).**

Polymorphisms of β_2-adrenergic receptor gene

Within the *ADRB2* gene, many single-nucleotide polymorphisms (SNPs) have been identified, the most common are 2 missense polymorphisms in the coding region of *ADRB2* (***Karam et al., 2013***). The first SNP caused by a change of adenine to guanine (A > G) at nucleotide 46 resulting in amino acid substitution from arginine to glycine at codon 16 (Arg16Gly or R16G; *rs1042713*). The second SNP is the change of cytosine to guanine (C > G) at nucleotide 79 causing the substitution from glutamine to glutamic acid at codon 27 (Gln27Glu or Q27E; *rs1042714*), as shown in figure (8). Each individual has two *ADRB2* genes, thus can be homozygote or heterozygote for a given polymorphism (***Hesse and Eisenach, 2008; Al-Rubaish, 2011***).

These two polymorphisms are located within the amino-terminal extracellular domain near the receptor's ligand-binding site (***Paiva et al., 2014***). They are known to alter the functional properties of the receptor and its behavior after agonist exposure. The main clinical interest in these polymorphisms lies in the possibility that they might determine the extent to which the receptor down-regulates in the airways and as such might modify bronchodilator responses (***Augusto et al., 2010***).

Ethnic difference in ADRB2 polymorphisms

Frequency analysis of the *ADRB2* alleles in different ethnic populations revealed the Gly_{16} and Gln_{27} to be the most frequent alleles, whereas, Arg_{16} and Glu_{27} are less frequently found in different population, with minor allele frequencies (MAF) of 30-40% and 40-50%, respectively (***Hegab et al., 2004; Leineweber and Heusch, 2009; Bernardez et al., 2010; Paiva et al., 2014***).

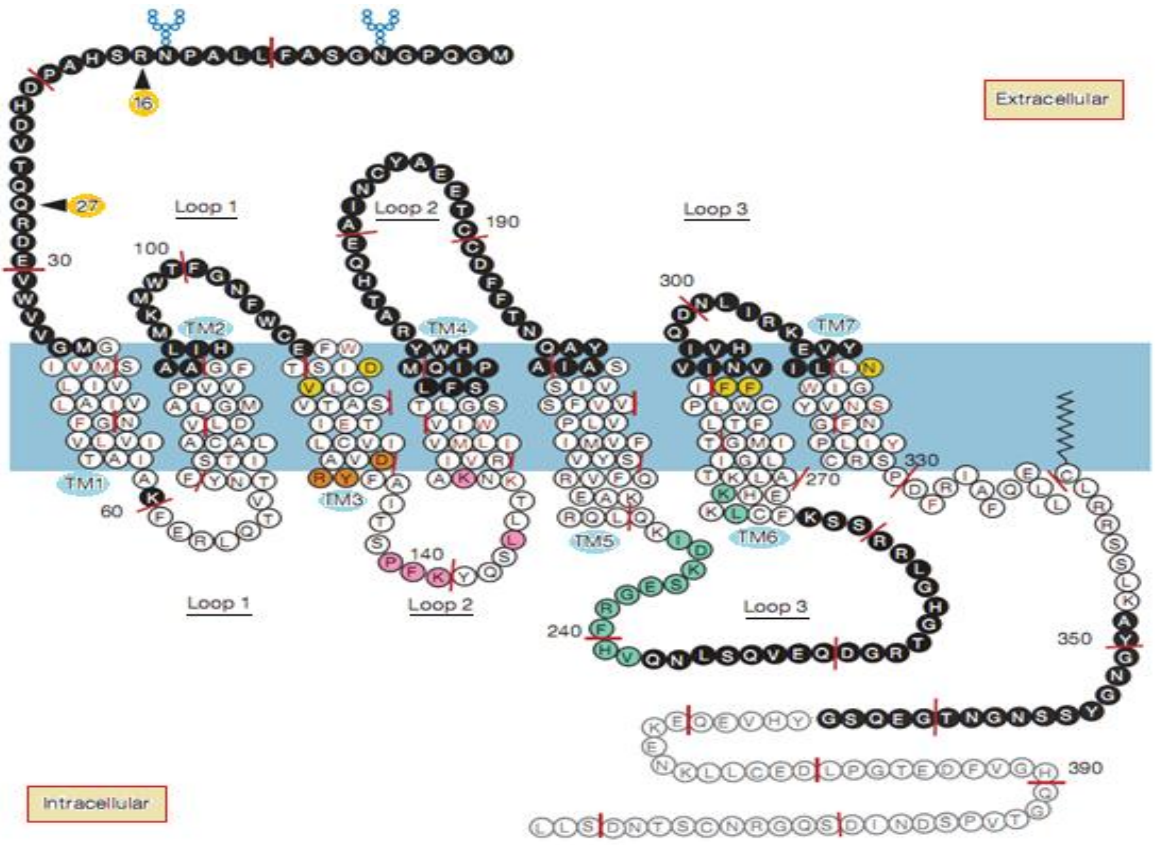


Figure (8). The human beta$_2$ adrenergic receptor polymorphisms. *Letters* within the circles are the primary amino acid sequence. *Red lines* indicate ten-amino-acid increments. *Black arrows* indicate site of SNPs at codon 16 and 27, Arg16Gly and Gln27Glu, respectively (***Rasmussen et al., 2007***).

Linkage disequilibrium of ADRB2 polymorphisms

Linkage disequilibrium (LD) exists between these two SNPs, resulting in common combination of polymorphisms (haplotypes): Glu_{27} is almost always associated with Gly_{16},

whereas Gln_{27} can coexist with either Arg_{16} or Gly_{16} (***Hegab et al., 2004; Leineweber and Heusch, 2009***).

Functional properties of β_2-adrenoceptor gene polymorphisms

The Arg16Gly and Gln27Glu polymorphisms do not alter the binding capacity of catecholamines to β_2-AR and AC activity. In addition, receptor synthesis rates and agonist-promoted internalization were not different between the receptors (***Small et al., 2003***). However, studies have demonstrated that these polymorphisms are strongly associated with the susceptibility of these receptors to the down-regulation phenomenon and functional desensitization. *In vitro*, Gly_{16} allele enhances agonist-induced down-regulation of the receptor number relative to the Arg_{16} polymorphism, while Glu_{27} allele enhances resistance to down-regulation when compared with the Gln_{27} variant. This difference in down-regulation may be due to alterations in receptor degradation after the internalization step (***Bernardez et al., 2010; Masuo and Lambert, 2011; Adriani et al., 2012***).

Interestingly, receptors with both Gly_{16} and Glu_{27} polymorphisms down-regulate to a similar extent to the Gly_{16} variant alone, thus showing that the influence of the Gly_{16} dominates over any protective effects of the Glu_{27} polymorphism. The rare $Arg_{16}Glu_{27}$ haplotype is the one that presents higher resistance to desensitization (***Bochdanovits et al., 2009; Al-Rubaish, 2011; Chung et al., 2011***). Moreover, studies demonstrate Arg_{16} allele to represent a condition with less ability to clear fluid from the lungs compared to the Gly_{16} variant; this may be related to a decrease in alveolar-capillary membrane conductance in this group (***Synder et al., 2008***).

ADRB2 polymorphisms and COPD

ADBR2 gene polymorphisms and human diseases

β_2-adrenergic receptors contribute to the regulation of many physiological functions. The clinical relevance of Arg16Gly and Gln27Glu polymorphisms in *ADRB2* gene has been explored in a range of disorders including respiratory illness, congestive heart failure, coronary heart disease, hypertension, cystic fibrosis, obesity, diabetes, colorectal carcinoma, Graves' disease, rheumatoid arthritis, atopic dermatitis, open-angle glaucoma, and polycystic ovary syndrome (***Gope et al., 2005; Maxwell et al., 2005; Inagaki et al., 2006; Kurabayashi et al., 2006; Roguedas et al., 2006; Sábato et al., 2008; Chu et al., 2009; Adriani et al., 2012***).

ADBR2 gene polymorphisms in obstructive airway diseases

Several studies have demonstrated that altered β_2-adrenergic receptor signaling due to the *ADBR2* polymorphisms for Arg16Gly and Gln27Glu could potentially affect airway smooth muscle contractility, mucociliary clearance, vascular endothelial permeability, and inflammatory cytokine production (***Hizawa et al., 2007; Thomsen et al., 2012***). These polymorphisms act as disease modifiers and are a determinant of responsiveness to β-agonist therapy (***Stephen, 2000; Bernardez et al., 2010***).

1. Arg16Gly polymorphism

Disease susceptibility

In studies examining susceptibility to obstructive airway diseases (asthma and COPD), the Arg_{16} homozygotes had increased risk of COPD and asthma (***Matheson et al., 2006; Yang et al., 2007***). However, other studies have produced conflicted results, showing that the distribution of genotype frequency of homozygote Arg_{16}/Arg_{16} in COPD patients was significantly lower than in healthy population (***Molfino, 2004; Hawkins et al., 2006; Vacca et al., 2009***). In addition, other studies reported that Arg16Gly polymorphism was not

associated with developing COPD (***Brøgger et al., 2006***), or with the susceptibility of asthma (***Augusto et al., 2010***).

Disease severity

Papatheodoroua et al (***2010***) showed a significant association between the Gly_{16} *ADRB2* polymorphism and the severity of COPD. Similarly, several studies indicated a correlation between the Gly_{16} genotype and nocturnal asthma, and steroid-dependent asthma (***Contopoulos-Ioannidis et al., 2005; Yin et al., 2006; Shigemitsu and Afshar, 2007; Augusto et al., 2010***). Other clinical studies have supported the possibility that the Gly_{16} form of the receptor is associated with markers of more severe asthma (***Johnson, 1998; Naghan et al., 2013***). In addition, ***Fukui*** et al. (***2006***) and ***Limsuwan*** et al. (***2010***) suggested that Gly_{16} may be associated with increased bronchial hyper-responsiveness (BHR) in asthmatic patients. On the contrary, other studies reported that homozygote patients with Arg_{16} receptors had increased risk of asthma exacerbations compared with Gly_{16} homozygotes (***Hawkins et al., 2006; Palmer et al., 2006; Yang et al., 2007***).

Pulmonary function parameters

Several association studies found that Arg_{16} homozygotes had lower lung function compared with Gly_{16} homozygotes, particularly in children/young adult asthmatics using salmeterol (***Hawkins et al., 2006; Palmer et al., 2006; Yang et al., 2007***). They also had less bronchodilatation after exercise compared with Gly_{16} homozygotes (***Synder et al., 2006***). This was in consistent with ***Zhang*** et al. (***2007***) who found that the Arg_{16} allele was associated with a decrease in FEV_1 and FVC in children with exposure to tobacco smoke during pregnancy, infancy and childhood.

This matched the finding of Arg_{16} being a risk genotype for poor response to β_2-agonists (salmeterol), although this was not replicated in other clinical trials using salmeterol with fluticasone (***Bleecker et al., 2006; Yang et al., 2007***). In contrast, other studies found no statistically significant difference in asthma control, FEV_1 or Peaked expiratory flow rate (PEFR) between genotypes of Arg16Gly polymorphism (***Silverman et al., 2003; Bleecker et al., 2006; Augusto et al., 2010***).

2. Gln27Glu polymorphism

Disease susceptibility

The potentially protective Glu_{27} allele has indeed been reported to be associated with decreased risk of childhood wheezing illness and decreased airway reactivity in asthma but it did not seem to influence nocturnal asthma (***Yang et al., 2007; Lee et al., 2009***). These findings may be because the Glu_{27} variant is resistant to down regulation *in vitro*, and it is possible that these individuals express higher β_2-receptor levels (***Thakkinstian et al., 2005***). The Gln_{27} allele, on the other hand, has been associated with elevated IgE levels and with an increased risk of childhood asthma and BHR (***Matheson et al., 2006; Yang et al., 2007***). However, ***Al-Rubaish*** (***2011***) demonstrated no association of Gln27Glu polymorphism with bronchial asthma.

Regarding COPD, the Glu_{27} polymorphism was not protective. A study upon Egyptian COPD patients found that Glu_{27} of the ADRB2 gene was associated with the development of COPD (***Hegab et al., 2004***). This may be confounded by its linkage disequilibrium with Gly_{16}. On contrast, other studies showed no significant difference in the frequency of Gln27Glu polymorphism between patients with COPD and healthy controls (***Ho et al., 2001; Molfino, 2004***).

Disease severity

Contopoulos-Ioannidis et al. (***2005***) reported a possible protective association between the Glu_{27} allele and asthma severity for subjects of Asian descent. ***Ho*** et al. (***2001***) and ***Molfino*** (***2004***) demonstrated that the patients with severe COPD were more likely to have Gln_{27} allele.

Pulmonary function parameters

A study on Chinese patients found that subjects with the Gln_{27} allele had lower FEV_1 percent of predicted. In contrast, the Lung Health Study (LHS) of current smokers found a protective effect of heterozygosity at position 27 against rapid decline in lung function in smokers (***Joos et al., 2003; Molfino, 2004; Matheson et al., 2006***). ***Martinez*** et al. (***1997***)

and ***Taylor*** (***2006***) reported no difference in treatment-related outcomes between the Gln_{27} and Glu_{27} genotypes. However, ***Matheson*** et al. (***2006***) reported that The Gln_{27} homozygotes had an increased risk of BHR.

3. Arg16Gly and Gln27Glu haplotypes

Disease susceptibility and severity

Several genetic association studies have investigated *ADRB2* haplotypes and risk of asthma, COPD, or related phenotypes with conflicting results. Most association studies revealed the Arg_{16}/Gln_{27} haplotype to be associated with more risk of developing COPD, asthma and bronchial hyper-responsiveness (BHR), and may be associated with more severe respiratory symptoms in middle-aged and older adults and frequent exacerbations (***Matheson et al., 2006; Hersh, 2010***). In contrast, studies showed that children with Gly_{16}/Glu_{27} haplotype (relative to all other *ADRB2* haplotypes) had a significantly lower risk for wheezing illness with a clear dose-response relationship (***Lee et al., 2009***).

Pulmonary function parameters

The Arg_{16}/Gln_{27} haplotype was also associated with a lower FEF_{25-75} percent predicted; a feature of small airways disease, which has been shown to progress to COPD (***Hersh, 2010***). The haplotype combination of Arg_{16}/Gln_{27} was associated with a reduced response to β_2-agonists *in vivo*, reduced BDR, and more predisposition to developing tachyphylaxis, highlighting the importance of examining haplotypes rather than studying single SNPs (***Matheson et al., 2006; Tellería et al., 2006; Hersh, 2010***).

On the other hand, two studies have found the Gly_{16}/Gln_{27} haplotype was protective against BHR, which is a known risk factor for chronic respiratory symptoms and mortality from COPD (***Augusto et. al., 2004; Ulbrecht et al., 2000***). Conversely, an Italian study found the Gly_{16}/Gln_{27} haplotype to be associated with an increased risk of BHR (***D'Amato et al., 1998***). Furthermore, the LHS found no association between any *ADRB2* haplotypes and bronchodilator response or non-specific BHR, or rate of decline in lung function (***Hersh, 2010***).

ADRB2 polymorphisms and drug response

β-agonists are the most common bronchodilators used to treat airflow limitation associated with obstructive airways diseases. Individual therapeutic responses to β-agonists differ, and can be influenced by several factors, including degree of baseline airway obstruction, ethnicity, and age. Therapeutic responses can also be affected by receptor desensitization produced by prolonged β-agonist use during regular therapy. The mechanisms responsible for heterogeneity in β-agonist responsiveness have not been completely elucidated; however, there is evidence that genetic variation in the *ADRB2* gene may be one important factor (***Hawkins et al., 2006***).

The Arg16Gly and Gln27Glu polymorphisms result in differential agonist-stimulated receptor down-regulation *in vivo* and *in vitro*, both without affecting agonist binding affinities or signaling activity. Receptors containing Gly_{16}/Gln_{27} and Gly_{16}/Glu_{27} had greater down-regulation after isoproterenol exposure than did receptors containing Arg_{16}/Gln_{27}, whereas Arg_{16}/Glu_{27} receptors exhibited no down-regulation. Thus these polymorphic variations in β_2-adrenoceptors may be important in determining their treatment response to β-agonist therapy in COPD patients (***Hegab et al., 2004; Hawkins et al., 2006; Hall, 2009***).

1. Acute bronchodilator response to short-acting β-agonists

Hawkins et al. (***2006***) and ***Chung*** et al. (***2011***) showed that individuals who were homozygous for Arg_{16} showed a greater bronchodilator response and higher predicted FEV_1 values compared with the Gly_{16} homozygotes, while Arg_{16}/Gly_{16} heterozygotes had intermediate response. The genetic effects of the Arg16Gly polymorphism were independent of the severity of airflow limitation, age, and smoking status. However, the bronchodilator responsiveness was not influenced by the variation of Gln27Glu polymorphism. In ***2010***, ***Cho*** reported that patients with the homozygous $Arg_{16}Gln_{27}$ haplotype pairs and those heterozygous for $Arg_{16}Gln_{27}/Gly_{16}Glu_{27}$ showed the highest bronchodilatation responses, while the patients with the homozygous $Gly_{16}Gln_{27}$ haplotype pairs showed the lowest response.

2. Clinical response to regular use of short-acting β-agonists

The effects of the *ADRB2* gene seem to depend on the duration of therapy. The decrease in the receptor number caused by long-term β_2-agonist exposure has been shown to be the result of decreased transcription, decreased mRNA stability, and degradation of the β_2-AR protein. In contrast, acute desensitization results from phosphorylation of the receptor and subsequent uncoupling of the receptor from Gs. Since both long-term and acute desensitization involve distinct mechanisms, the influence of *ADBR2* gene polymorphisms might not necessarily be the same (***Moore et al., 2000***).

In Arg_{16} homozygotes, the receptors are less susceptible to endogenous agonist-induced down-regulation, which would explain the superior response to acute β_2 agonist therapy compared with Gly_{16} homozygotes. However, with prolonged or chronic ADRB2 agonist therapy agonists in children, young adults, and adult patients Arg_{16} receptors may undergo further down-regulation, giving rise to poorer response to SABA in the presence or absence of concurrent inhaled corticosteroids, reduced lung function, more frequent and prolonged exacerbations, more adverse effects and clinical tachyphylaxis, which is defined as a waning of effectiveness of an agonist over time during repetitive administration (***Hawkins t al., 2006; Hall, 2009; Cho, 2010***).

On the contrary, the Gly_{16} receptors are maximally down-regulated by endogenous catecholamines, and therefore show reduced response on first acute exposure to exogenous β_2 agonist, with no scope for further down-regulation or tachyphylaxis with chronic exposure (***Chung et al., 2011***). Gly_{16}/Gly_{16} subjects with asthma receiving regular, short-acting β-agonist therapy (albuterol) experienced improvement in peak flow rates and fewer daily asthma symptoms (***Hawkins et al., 2006***).

Regarding the Gln27Glu polymorphism and drug response, although there was no difference in treatment-related outcomes between the Gln27 and Glu27 genotypes when SNPs were analyzed individually (***Chung et al., 2011***), there was a clear relation between haplotypes which included Glu_{27} allele and good response to β_2 agonists *in vivo*, as well as increased messenger RNA levels and gene expression *in vitro*. On the other hand,

haplotypes that included Gln_{27} had overall poorer response to β_2 agonists and lower expression levels. This effect was altered by the over-dominant protective effect of Glu_{27} allele in heterozygote haplotypes (***Thakkinstian et al., 2005***).

3. Clinical response to regular use of long-acting β-agonists

With long-acting β_2 agonists, the overall clinical benefits may be worse for Arg_{16} than for the Gly_{16} genotype. Patients with Arg_{16}/Arg_{16} genotype had decreased responses to regularly used long-acting $beta_2$-agonist (LABA) in the presence or absence of concurrent ICS than did those who used them as needed, with greater risk of deterioration and more frequent disease exacerbations. In contrast, in patients with Gly_{16}/Gly_{16} genotype, regular usage of LABA therapy yield greater improvement in airway function when compared with as-needed β_2 agonist usage (***Isada et al., 2008; Cho, 2010***). However, this was inconsistent with a recent study by ***Rabe*** et al. (***2014***) who reported that COPD patients with the Arg_{16}/Arg_{16} genotype had better exacerbation outcomes in response to salmeterol than Gly_{16}/Gly_{16} and Arg_{16}/Gly_{16} genotypes.

In vitro, corticosteroid therapy up-regulates the β_2-adrenergic receptor, thereby, generating a drug-drug interaction with β_2 agonists. This interaction has the potential to mask the genotypic effects of *ADRB2* polymorphisms during concomitant LABA and ICS therapy (***Ortega et al., 2007; Sábato et al., 2008***). The hypothesis that genetic polymorphisms might predispose an individual to disease, or alter the clinical progression of disease, is important not only from a prognostic standpoint but also in the context of individual responses to treatment. β_2 agonists are effective and safe in most patients with COPD, but they may be less effective or potentially harmful in some. Therefore, it is mandatory to match individualized treatments to specific genotypes in a way that minimizes side effects while improving therapeutic outcomes (***Hizawa, 2009***).

SUBJECTS

Type of Study

A case control study conducted to assess the genetic association between two different polymorphisms of *ADRB2* gene and chronic obstructive pulmonary disease in Egyptian patients.

Target population and site

The study included two groups; COPD patients and controls. All subjects gave a written consent prior to participation. Study protocol was approved by the Institutional Research and Medical Ethics Committees of Cairo University.

Group (1): COPD cases

Sixty one unrelated Egyptian patients with COPD were enrolled in the study. They were recruited from Abasseyiah Chest Hospital, Cairo, Embaba Chest Hospital, Cairo, and Port-Said Chest Hospital, Port-Said, Egypt.

Inclusion Criteria

1- COPD patients were diagnosed and classified according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (***Mervyn, 2009***), [**Appendix I**].
2- Egyptian patients with any age or gender.

Exclusion Criteria

1- Patients with clinical or radiographic findings consistent with any other chest disease, or heart failure.
2- Patients with severe exacerbation of COPD who require non-invasive or invasive mechanical ventilation according to the GOLD guidelines (***Mokry et al., 2008***).
3- Patients who had relatives enrolled in the study.

Group (2): Controls

The control group included 54 apparently healthy volunteers, with no history of chronic illness.

Inclusion criteria

1- Egyptian healthy individuals matched by age and gender to cases in group 1.

2- Normal spirometry.

Exclusion criteria

1- Individuals with history or spirometric findings consistent with COPD, or any other respiratory system diseases.

2- Individuals who had relatives enrolled in the study.

MATERIALS

(A)Clinical and radiological evaluation of the study population

All study subjects underwent the following:

- A full medical history; including personal, present, past and family medical history, using the structured interview, [**Appendix II**].
- History of smoking status and intensity using Brinkman smoking index (Brinkman index: number of cigarettes smoked per day × number of years of the habit) (***Brinkman and Coates , 1963***).
- Body mass index (BMI) using the following equation BMI= weight (kilogram)/ height (meter) ² (***WHO, 2004***). BMI > 30 Kg/m² was considered obese (***Bedogni, 2001***).

COPD patients were examined for the following:

- Severity of disease based on GOLD's criteria.
- Severity of dyspnea based on Modified Medical Research Council (mMRC) scale (***Stenton, 2008***), [**Appendix III**].
- The Arabic version of COPD Assessment Test (CAT) was used to assess the impact of COPD on health-related quality of life (***Jones et al., 2012***), [**Appendix IV**].
- Exacerbation episodes of disease during the past year (***Mokry et al., 2008***).

- History of current pharmacological treatment in COPD patients (oral and inhaled corticosteroids, bronchodilators, long-term oxygen therapy).
- Plain Chest X ray, postero-anterior view to exclude other causes of airflow limitation.

(B) Pulmonary function tests:

Spirometry was performed in 43 patients and 35 controls in accordance with the guidelines of the American Thoracic Society/European Respiratory Society (ATS/ERS) consensus to evaluate their baseline pulmonary function (***Soriano et al., 2013***). In addition, post-bronchodilator forced spirometry was repeated in COPD patients (15 minutes) after administering of inhaled salbutamol (Ventolin; *GlaxoSmithKline*).

Equipment

An electronic spirometer (*BTL-08 Spiro Pro system; BTL*) was used. Temperature and barometric pressure are measured automatically by the device.

Figure (9). Electronic spirometry.

Baseline pulmonary function

Test procedures (Miller et al., 2005b)

There were three distinct phases to the maneuver, as follows: a) maximal inspiration; b) a ‘‘blast’’ of exhalation; and c) continued complete exhalation to the end of test.

1) Preparing the spirometer
   - Check the spirometer calibration.
   - Assemble the components according to the manufacturer’s instructions (i.e. tubing, connectors, flow-sensors, valves and adapters).
2) Preparing the patient
   - Carry out infection control measures prior to testing, particularly hand washing for both patient and personnel performing spirometry.
   - Document medication use, recent illness, smoking, etc.
   - Measure weight and height without shoes.

- Withheld bronchodilator medications prior to testing: stop short-acting inhaled drugs (SABA or ipratropium bromide) for 6 hours, long-acting inhaled drugs (LABA) or oral aminophylline for 12 hours and inhaled long-acting muscarinic antagonist (LAMA) for 24 hours before spirometry (***Mokry et al., 2008***).
- Smoking or any beverages containing xanthines is ceased for > 1 hour prior and throughout test.
- Verify the patient's identity (full name, gender, and date of birth).

3) Instruct and demonstrate the maneuver to the subject, to include
   - Correct posture with head slightly elevated.
   - Position of the mouthpiece.
   - Inhale rapidly and completely.
   - Exhale with maximal force.
   - The pitfalls that could render the test unacceptable.
4) Perform maneuver (closed circuit method)
   - Have subject assume the correct posture (sitting with upright posture, legs uncrossed and both feet flat on the floor).
   - Attach nose clip, place mouthpiece in mouth and close lips around the mouthpiece.
   - Inhale completely and rapidly with a pause of <1s at total lung capacity (TLC).
   - Exhale maximally until no more air can be expelled while maintaining an upright posture.
   - Verbal encouragement and instructions to have a continuous smooth exhalation till the end of test.
   - Repeat instructions as necessary, coaching vigorously.
   - Repeat for a minimum of three maneuvers; no more than eight are usually required.
   - Check test repeatability and perform more maneuvers as necessary.

Acceptability criteria (Miller et al., 2005b)

Individual spirograms are ''acceptable'' if

1) They are free from artifacts that interfere with the measurement of accurate results
   - Cough during the first second of exhalation
   - Glottis closure or excessive hesitation that influences the measurement

- Early termination or cut-off
- Effort that is not maximal throughout
- Leak
- Obstructed mouthpiece

2) They have good starts
- Extrapolated volume 5% of FVC or 0.15 L, whichever is greater

3) They show satisfactory exhalation
- Duration of ≥6 s or a plateau in the volume–time curve or
- If the subject cannot or should not continue to exhale.

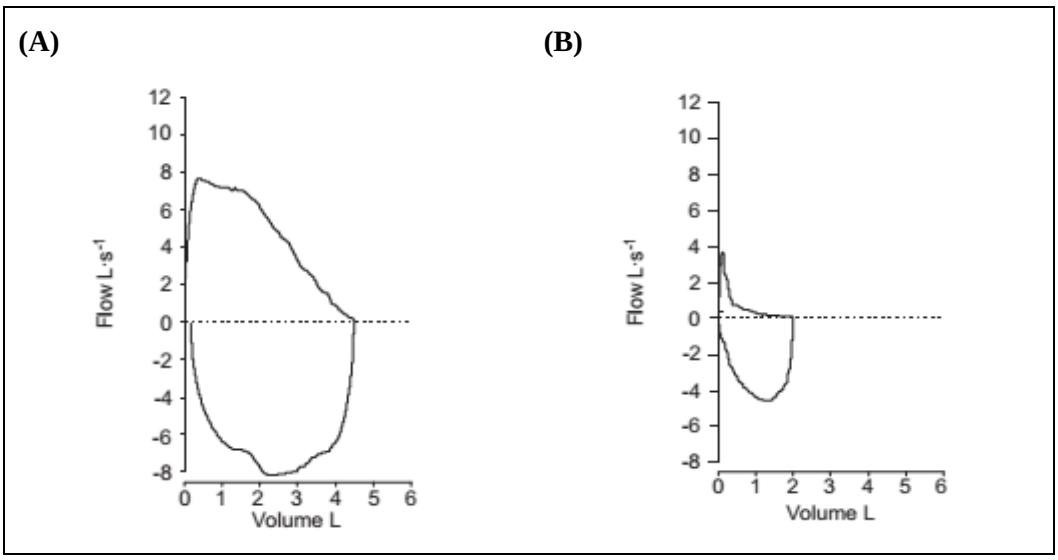


Figure (10). Flow-volume curve in normal and COPD individuals. (A) Flow–volume loop of a normal subject. (B) Severe airflow limitation in a subject with chronic obstructive pulmonary disease (***Miller et al., 2005b***).

Between-maneuver evaluation

1) After three acceptable spirograms have been obtained, apply the following tests
- The two largest values of FVC and FEV_1 must be within 0.150 L of each other. For values of FVC ≤ 1L, the difference should be less than 0.1L.

2) If both of these criteria are met, the test session may be concluded, Figure (10).

3) If both of these criteria are not met, continue testing until
- Both of the criteria are met with analysis of additional acceptable spirogram or
- A total of eight tests have been performed or

- The patient/subject cannot or should not continue

4) Save, as a minimum, the three satisfactory maneuvers.

Reversibility testing (*Miller et al., 2005b*)

To ensure good delivery of drug to the bronchial tree and avoid, as much as possible, faulty use of Metered-Dose Inhaler by the patients, we used valve-holding chamber (spacer). After a gentle and incomplete expiration, a 100 µg dose of inhaled salbutamol (Ventolin; *GlaxoSmithKline*) is inhaled in one breath to TLC from a valved spacer device. The breath is then held for 5–10 s before the patient exhales slowly. Four separate doses (total dose 400mg) are delivered at 30-s intervals. The following steps were undertaken. 1) The drug was administered in the dose and by the method indicated for the test. 2) After a duration of 10-15 min., the subject has three acceptable tests of FEV1, FVC and PEF recorded as described previously.

(A) Determination of *ADRB2* gene polymorphism

Lab work was performed in the Molecular Biology Lab of the Biotechnology Research Center, Suez Canal University. Four steps were performed: (1) specimen collection, (2) DNA extraction, (3) assessment of the concentration and purity of the extracted DNA, and finally (4) quantitative real-time PCR and data analysis.

1) Blood sample collection

Three milliliters of whole blood were obtained from healthy volunteers or patients with COPD, and placed in vacutainer tube containing Lithium Heparin 68 IU (*Becton Dickinson Plymouth*). Blood was preserved at -20 degrees Celsius until processed for deoxyribonucleic acid (DNA) extraction.

2) Genomic DNA extraction

DNA extraction from blood leukocytes was done using QIAamp DNA Blood Mini kits (*QIAgen, Clinilab Co., Egypt, Catalog no. 51104)*, according to the manufacturer's instructions (***Jung et al., 2006***).

Preparation of reagents

1. *Preparation of QIAGEN Protease stock solution (lysis buffer):* 1.2 ml protease solvent was pipetted into the vial containing lyophilized QIAGEN Protease. Then it was stored in aliquots.
2. *Preparation of AL Buffer:* the buffer was mixed thoroughly by shaking before using each time. It has been kept at room temperature.
3. *Preparation of wash Buffers AW_1 and AW_2:* they were supplied as a concentrate. Ethanol (100%) was added, before the first time of use, as shown in Table (11), and kept at room temperature.

Table (11). Preparation of buffer AW_1 and AW_2.

Buffer	Concentrate (ml)	Ethanol (ml)	Final volume (ml)
AW1	19	25	44
AW2	13	30	43

QIAamp spin procedures

1. QIAGEN Protease was brought down from freezing temperature to room temperature, and 20 µl was pipetted into the bottom of an eppendorf tube.
2. 200 µl of blood sample was added to the tube for cell lysis.
3. 200 µl of Buffer AL was added to the sample (the buffer was incubated first at 56°C for 1 min to dissolve precipitate before use).
4. Samples were incubated at 56°C in a heating block for 10 min then centrifuged briefly to remove drops from the inside of the lid. Then 200 µl of ethanol (96–100%) were added, and mixed by pulse-vortexing for 15 sec.
5. Samples were then centrifuged briefly, and applied carefully into a 2 ml collection tube (QIAamp Spin Column), this was followed by centrifugation at 13000 rpm for 1 min.

6. The QIAamp Spin Column was then placed in another clean collection tube and the original collecting tube was discarded. 500 µl Buffer AW_1 was added, and recentrifuged at 13000 rpm for another 1 min.
7. Step 6 was repeated but using another wash buffer (500 µl Buffer AW_2) so as to ensure complete removal of any residual contaminants and centrifuging at 14000 rpm for 3 min, Figure (11).
8. The QIAamp Spin Column was further placed in another clean collection tube and the original collecting tube was discarded with the filtrate, and recentrifuged at 14000 rpm for 1 min.
9. The QIAamp Spin Column was then placed in a clean 1.5 ml eppendorf tube, in which 200 µl of Buffer AE were added.
10. This eluted DNA was incubated at room temperature for 5 min, then centrifuged at 13000 rpm for 1 min.
11. A portion of purified DNA (10 µl) was aliquoted for quantitative and qualitative analysis. The remaining DNA was stored at –20°C until used in polymerase chain reaction (PCR) analysis.

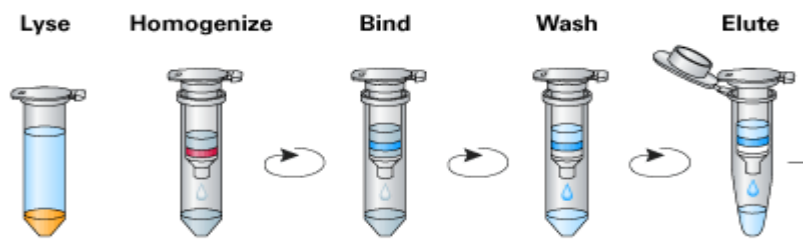


Figure (11). Procedural overview of DNA purification (*www.Qiagen.com*)

3) Determination of extracted DNA integrity, concentration and purity

Concentration and purity of the extracted DNA of each sample were measured by NanoDrop ND-1000 (*NanoDrop Tech., Inc. Wilmington, DE, USA*), and its integrity was determined by electrophoresis, at 90 Volts, on a 0.8% agarose gel (*Promega, Catalogue no. V3121*), containing 0.5 µg/ml of ethidium bromide (***Roughley et al., 2006***).

4) *ADRB2* polymorphism genotyping

Genotyping of the single nucleotide polymorphisms at positions 16 (Arg16Gly; *rs1042713*) and 27 (Gln27Glu; *rs1042714*) was performed using real-time polymerase chain reaction (RT-PCR) technology, using TaqMan® genotyping assays for both SNPs (*Applied Biosystem, assay ID C__2084764_20 and C__2084765_20*), to detect the presence of one or both alleles in each sample (***Augusto et al., 2010; Al-Rubaish, 2011***).

Each assay contained both the forward and reverse PCR primers, VIC®/FAM-labeled probes, Table (12). SDS (Software-Defined Storage) Software v1.4 (*PN 4374432*) would detect the magnitude of the fluorescence signal emitted if the probe bind to the complementary DNA and translate that into the presence or absence of the corresponding allele. Thus classifying the samples into homozygotes (containing one allele) or heterozygotes (containing both alleles).

Thus for Arg16Gly polymorphism, possible genotypes were AA, AG, and GG. The probe for allele A (Arginine) was labeled with VIC® dye; the probe for allele G (Glycine) was labeled with FAM™ dye. For Gln27Glu polymorphism, possible genotypes were CC, CG, and GG. The probe for allele C (Glutamine) was labeled with VIC® dye; the probe for allele G (Glutamic acid) was labeled with FAM™ dye.

Table (12). TaqMan® genotyping assays for tested *ADRB2* SNP (Appliedbiosystem.com)

Assay ID	SNP ID	SNP location	SNP type	Codon change (VIC/FAM)	Amino acid change
C_2084764_20	*rs1042713*	**285**	**Transition substitution**	**AGA, GGA**	**R16G**
C_2084765_20	*rs1042714*	**318**	**Transversion substitution**	**CAA, GAA**	**Q27E**

Procedure

A. Setting up the reaction plate

- **Preparing reaction mix**

1) The number of reactions to be performed for each assay was calculated. Two no-template controls (NTC) were included in each run.

2) The total volume of each component needed for all wells was calculated, using table (13).
3) The bottle of TaqMan Universal PCR Master Mix (2x) was gently swirled to resuspend.
4) The 40x SNP genotyping assay was diluted to a 20x working stock with DNase-RNase-free water, mixed vigorously using a vortex and briefly centrifuged.
5) The required total volumes of TaqMan Universal PCR Master Mix and 20x SNP Genotyping Assay were pipetted into 2 microcentrifuge tubes (for the 2 assays).
6) The tubes were capped (*Applied Biosystem, catalogue no 4323032*) and inverted several times to mix. Then briefly centrifuged to spin down the contents and to eliminate any air bubbles from the reaction mix.

Table (13). The total volume of each component of PCR reaction mix.

Reaction components	Volume/well (25µL volume reaction)
Taqman® Universal PCR Master Mix, No AmpErase UNG (2x)	12.5µL
20 x TaqMan® SNP Genotyping Assay Mix	1.25µL
20 ng genomic DNA was diluted to 11.25µL withDNase-RNase-free water	11.25µL
Total	25µL

- **Preparing DNA**

1) Frozen DNA samples were thawed, vigorously mixed, and then centrifuged briefly.
2) Genomic DNA samples were pipetted into RNase- and DNase-free MicroAmp® 0.2 mL Optical 8-tube strips (*Applied Biosystem, catalogue no 4316567*) to deliver a final mass of 20 ng per tube.
3) NTC contained 11.25 µL of DNase- RNase-free water without DNA.

- **Adding the reaction mix**

1) The reaction mix was added to the prepared DNA template. The tubes were capped with MicroAmp® Optical Cap Strips (*Applied Biosystem, catalogue no 4323032*), and mixed thoroughly by inversion and briefly centrifuged.
2) All the wells were inspected for uniformity of volume and absence of air bubbles. The 8-strip tubes were kept on ice.

B. Allelic Discrimination Plate Read and Analysis

- **Performing a pre-read run**

A pre-read run recorded the background fluorescence of each well of the AD plate document before PCR. During the post-read run, the pre-read fluorescence was subtracted from the post-read fluorescence to account for pre-amplification background fluorescence, ensuring accurate results.

- **Performing the amplification run**

The thermal cycling conditions were specified as shown in Table (14). The reaction volume was adjusted to 25 µl. The reaction plate was loaded into the thermal cycler, then, the run was started.

Table (14): Thermal cycling conditions.

Steps	Temperature	Time	No. of cycle
Initial step	95°C	10 min	One
Denaturation	92°C	15 sec	40 cycles
Annealing/ Extension	60°C	1 min	

- **Performing an allelic discrimination post-read run and evaluating results**

The SDS software plotted the results of the allelic discrimination run on a scatter plot of allele X versus allele Y. Each well of the 96-well reaction plate was represented with an (Undetermined) on the plot. Manual allele calls were made then converted to genotypes. The clustering of points can vary along the horizontal axis

(allele X), vertical axis (allele Y), or diagonal (allele X/allele Y), Figure (12). This variation is due to differences in the extent of reporter dye fluorescent intensity after PCR amplification.

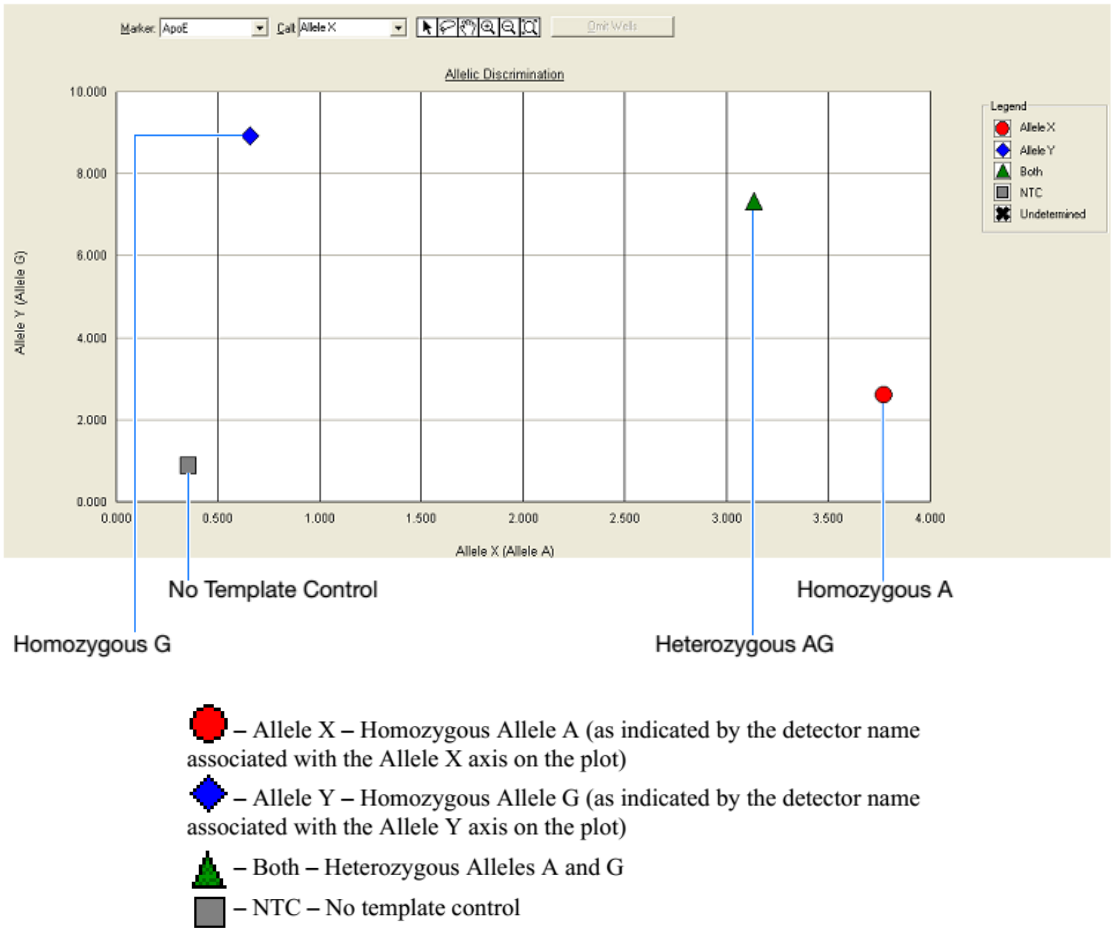


Figure (12). Allelic discrimination plot (*Applied Biosystem*).

SPECIAL PRECAUTIONS

Considerations to avoid possible risk include:

- To avoid cross infection while using spirometry, each patient used his own disposable mouth piece and his own bronchodilator inhaler. In addition, monitoring for any potential side-effects of salbutamol (as tachycardia, tremors, etc.) was considered.
- Venipuncture, which is a minimally invasive procedure, was done with caution regarding to infection control measures, and all blood samples were handled as if being infectious.

STATISTICAL ANALYSIS

Statistical analysis was carried out using the Microsoft® Excel 2010 and the *"Statistical Package for the Social Sciences (SPSS) for windows"* software, version 20. Odds ratios (OR) with a 95% confidence interval (CI) were calculated. Descriptive statistics were represented as mean ± standard deviation (SD) for quantitative variables and percentage for qualitative variables. Differences between COPD patients and healthy controls were tested using analysis of variance (ANOVA), Student '*t*' or Chi-square (χ^2) test. *P*-value was considered statistically significant below 0.05.

The allele frequency within each group was determined as the number of occurrences of an individual allele divided by the total number of alleles. The carriage rate was calculated as the number of individuals carrying at least one of the investigated alleles divided by the total number of individuals in each group (***Blakemore et al., 1996; Jaana et al., 2002***).

Using allele frequencies observed in the study population, Hardy-Weinberg equilibrium (HWE) was used to calculate the expected genotype frequencies among patient and control groups, using the following equation ($a^2 + b^2 + 2ab = 1$), in which *a* and *b* are the frequencies of each allele (***Jalba et al., 2008***). χ^2 test was applied to compare the expected versus observed distribution of genotypes (***Hegab et al., 2005***).

Response to salbutamol was expressed in three ways: absolute change in FEV_1 [BDRABS = postbronchodilator FEV_1 − prebronchodilator FEV_1], change in FEV_1 as a percent of baseline FEV_1 [BDRBASE = ((postbronchodilator FEV_1 − prebronchodilator FEV_1) / prebronchodilator FEV_1) × 100], and change in FEV_1 as a percent of predicted FEV_1 [BDRPRED = ((postbronchodilator FEV_1 − prebronchodilator FEV_1) / predicted FEV_1) × 100] (***Hizawa et al., 2007; Kim et al., 2009***).

RESULTS

CHARACTERISTICS OF THE STUDY GROUPS

A total of 61 patients and 54 controls were enrolled in our study. Both groups were age and sex matched as the median age for patients was 62.13 (43 -69) *versus* 60.7 (30-89) in controls. Demographic features of participants are shown in Table (15). There was a significant difference between COPD patients and healthy individuals in respect to their smoking status and intensity ($p<0.001$).

Table (15). Demographic data of the study groups.

Variables		COPD (n=61)		Controls (n=54)		P value
Mean age, years		62.0	± 6.0	60.91	± 9.9	0.46
Age categories, (%)	Less than 40	0	(0)	2	(3.7)	0.24
	40-59 years old	15	(24.6)	16	(29.6)	
	60 or more	46	(75.4)	36	(66.7)	
Male gender, (%)		60	(98.4)	51	(94.4)	0.25
Mean weight, Kg		65.2	± 12.5	68.4	± 15.6	0.21
Mean BMI, Kg/m^2		23.1	± 4.5	24.2	± 4.0	0.20
BMI categories, (%)	≤18.5	7	(11.5)	4	(7.4)	0.31
	>18.5-25	36	(59.0)	25	(46.3)	
	>25-30	13	(21.3)	19	(35.2)	
	>30	5	(8.2)	6	(11.1)	
Smoking status, (%)	Smoker	27	(44.3)	19	(35.2)	**<0.001 ***
	Ex-smoker	33	(54.1)	3	(5.6)	
	Non-smoker	1	(1.6)	32	(59.2)	
Brinkman smoking index		1458	± 791	610	± 327	**<0.001 ***

Values are shown as mean ± standard deviation or as number (percentage). COPD, chronic obstructive pulmonary disease; BMI, body mass index; Brinkman smoking index, number of cigarettes per day times the number of years. Two-sided Chi-square (χ^2), Student t test, and Mann-Whitney U tests were used.

Clinical characteristics of COPD patients

The majority of COPD patients (75%) enrolled in the study had high degree of dyspnea; Grades ≥3 according to the mMRC scale, Figure (13). By using CAT score for symptom assessment, 40% of the patients had high levels of health status impairment, Figure (14). Mean mMRC and CAT scores were 3.1 ± 0.8 and 23.3 ± 7.9, respectively. Most of the patients (62.5%) experienced COPD exacerbations (≥2 times) in the previous year, Figure (15). Regarding their regular medications, 31% of patients are only taking SABA, 62.5% had adjuvant inhaled corticosteroids, and none had long-term oxygen therapy, Figure (16).

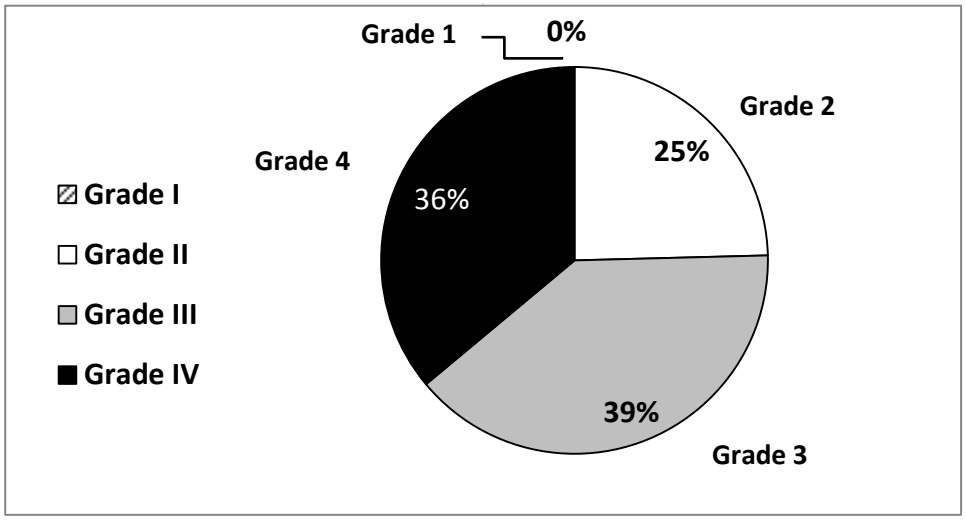


Figure (13). Severity of dyspnea of COPD patients (n=61). mMRC scale, (Modified Medical Research Council) scale for dyspnea severity was used.

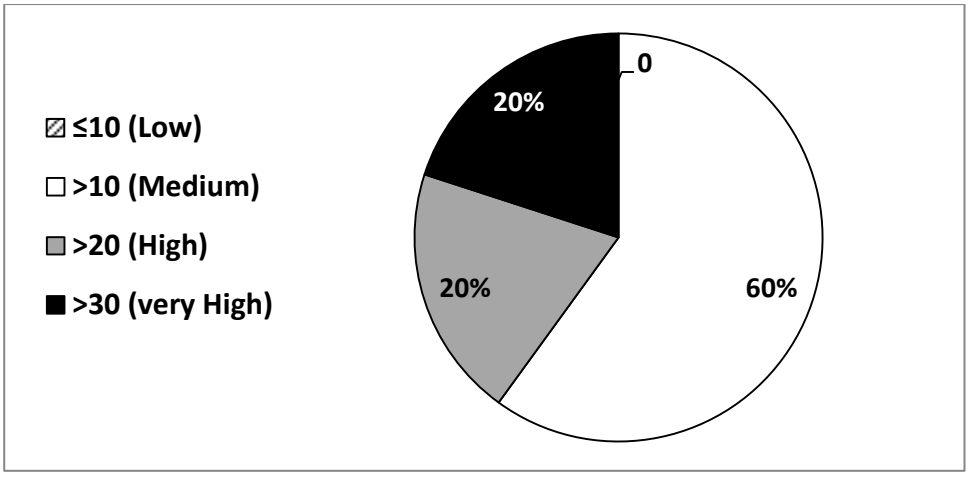


Figure (14). CAT scoring of COPD patients (n=61). CAT, COPD Assessment Test for estimating impact level of COPD on health status.

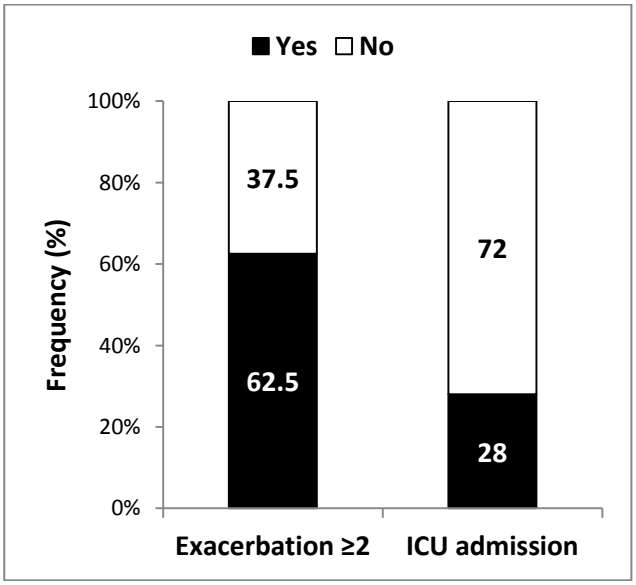


Figure (15). Frequency of exacerbation and ICU admission of COPD patients in the last year (n=61).

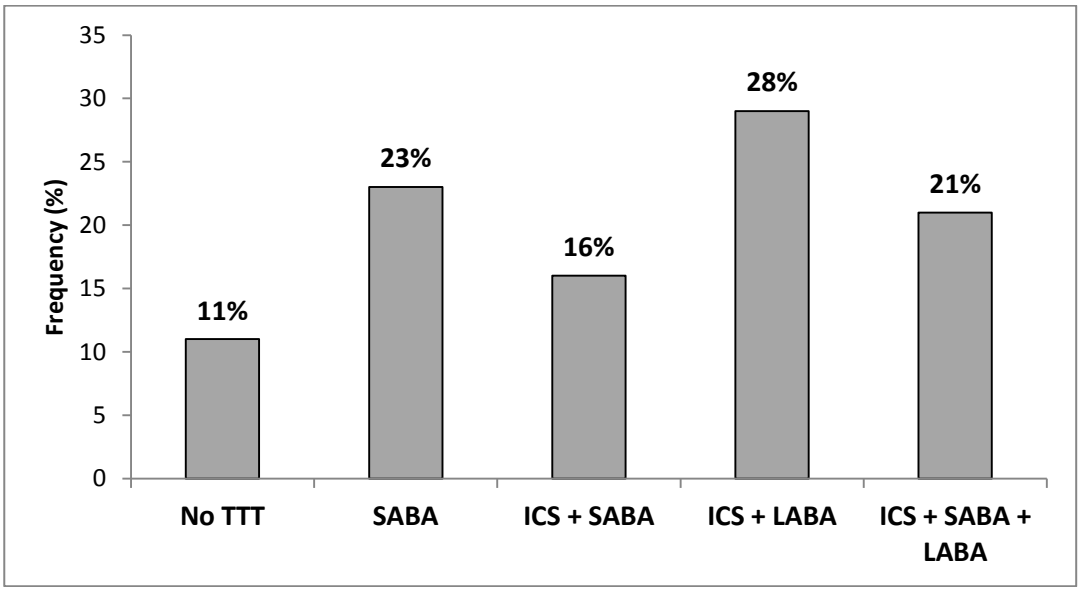


Figure (16). Regular medications used by COPD patients (n=61). TTT, treatment; SABA, short-acting β_2-agonist; LABA, Long- acting β_2-agonist; ICS, inhaled corticosteroids.

Functional parameters of the study groups

Using the Global Initiative for Chronic Obstructive Lung Disease (GOLD) staging system, most patients had advance disease: GOLD 1, none; GOLD 2, 9.3% (4 patients); GOLD 3, 53.5% (23 patients); and GOLD 4, 37.2% (16 patients), Figure (17). In COPD patients, mean baseline FEV_1 was 0.95±0.4 L (32.9±12.9% predicted) and forced vital capacity (FVC) was 1.78±0.65 L (48.6±16.4% predicted). Salbutamol induced an improvement of 51.6±14.8 mL (34.7±13.7% predicted) in FEV_1 and 79.5±12.1 mL in FVC, with a bronchodilator response (BDR) of 5.4±4.1 mL (1.85±1.6% predicted), Table (16).

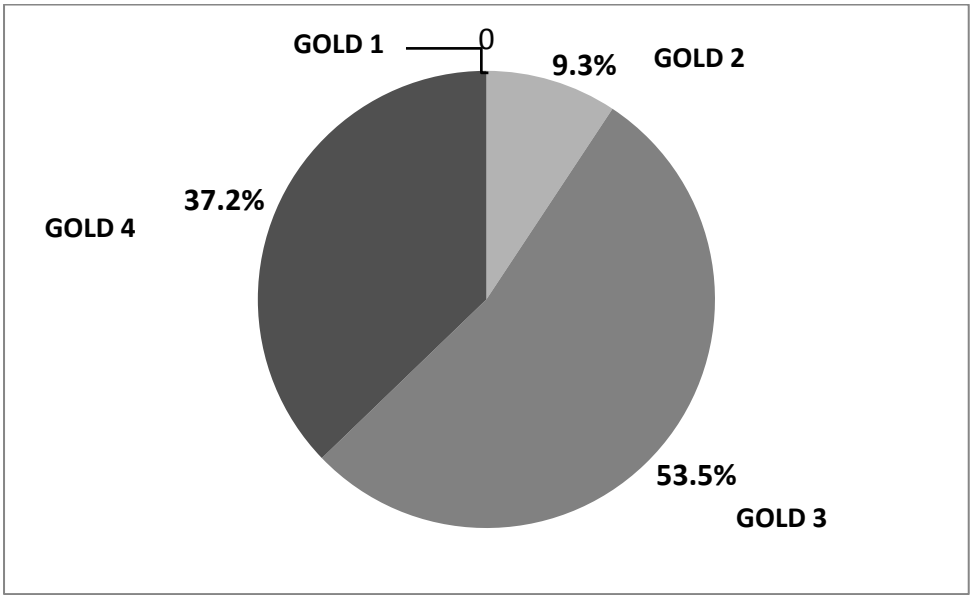


Figure (17). GOLD classification of airflow limitation in COPD patients (n=43). GOLD stage, Global Initiative for Chronic Obstructive Lung Disease criteria for disease staging.

Table (16). Pulmonary function parameters of the study groups.

Variables	COPD (n=43)	Controls (n=35)	P value
Pre-bronchodilator			
FEV_1 (% predicted)	32.9 ± 12.9	91.6 ±13.7	**<0.001***
FVC (% predicted)	48.6 ± 16.4	86.4 ±12.1	**<0.001***
FEV_1/FVC	53.2 ± 7.8	85.1 ±6.7	**<0.001***
Post-bronchodilator			
FEV_1 (% predicted)	34.7 ± 13.7		
FEV_1/FVC	53.6 ± 8.4		
BDR			
BDRABS (mL)	51.6 ± 45.0		
BDRPRED (%)	1.9 ± 1.6		
BDRBASE (%)	5.4 ± 4.1		

Values are shown as mean ± standard deviation. Spirometry taken before bronchodilatation (pre-BD) and after bronchodilatation (post-BD). FEV_1, Forced expiratory volume in the first second; FVC, forced vital capacity; PEF, peak expiratory flow; BDRABS, absolute change in FEV_1 [= post-BD FEV_1 – pre-BD FEV_1]; BDRBASE, change in FEV_1 as a percent of baseline FEV_1 [= ((post-BD FEV_1 – pre-BD FEV_1) / pre-BD FEV_1) × 100]; BDRPRED, change in FEV_1 as a percent of predicted FEV_1 [= ((post-BD FEV_1 – pre-BD FEV_1) / predicted FEV_1) × 100]. Mann-Whitney U test has been used.

MOLECULAR ANALYSIS OF *ADRB2* POLYMORPHISMS

Single SNP analysis

In the current study, we tested two SNP polymorphisms within the N-terminal domain of *ADRB2* gene; Arg16Gly (R16G; *rs1042713*) and Gln27Glu (Q27E; *rs1042714*). SNP analysis showed that the allele frequencies of Arg_{16} and Gly_{16} in our study population were 0.37 and 0.63, while that of Gln_{27} and Glu_{27} alleles were 0.67 and 0.33, respectively, Figure (18). Correspondingly, Arg_{16}/Gly_{16} was the most frequent genotype among the whole study population (60%), whereas, the most frequent genotypes in Gln27Glu polymorphism were Gln_{27}/Gln_{27} and Gln_{27}/Glu_{27} representing 45% and 49%, respectively, Figure (19).

Table (17) shows the genotype frequencies and carriage rates of *ADRB2* polymorphisms in COPD patients and healthy controls. In Arg16Gly polymorphism, Arg_{16}/Arg_{16} genotype was 4.1 times more likely to develop COPD than their counterparts (p=0.02). Carriage of the Gly_{16} allele was significantly lower in COPD patients compared to controls (94.4% in controls *versus* 80.3% in COPD patients; *P*=0.025), with odds ratio showing protective effect. Whereas, in Gln27Glu polymorphism, individuals with Gln_{27}/Gln_{27} were 3.4 times more susceptible to have COPD (*p*=0.001), while heterozygote individuals with Gln_{27}/Glu_{27} genotype showed protective effects against COPD risk. The frequency of Glu_{27} allele was significantly lower in patients with COPD compared to controls (24.6% *versus* 37.0%; *P*= 0.04), Figure (20). Carrying the Glu_{27} allele conferred protection against developing COPD, with OR (95% CI) of 0.292 (0.135-0.635), *p*=0.002). Stratified analysis by smoking shows no relation between smoking status and genotypes (Table 18). The frequencies of *ADRB2* genotypes among the COPD patients and controls were found in accordance with those expected by the Hardy Weinberg equilibrium ($P > 0.05$), Table (19).

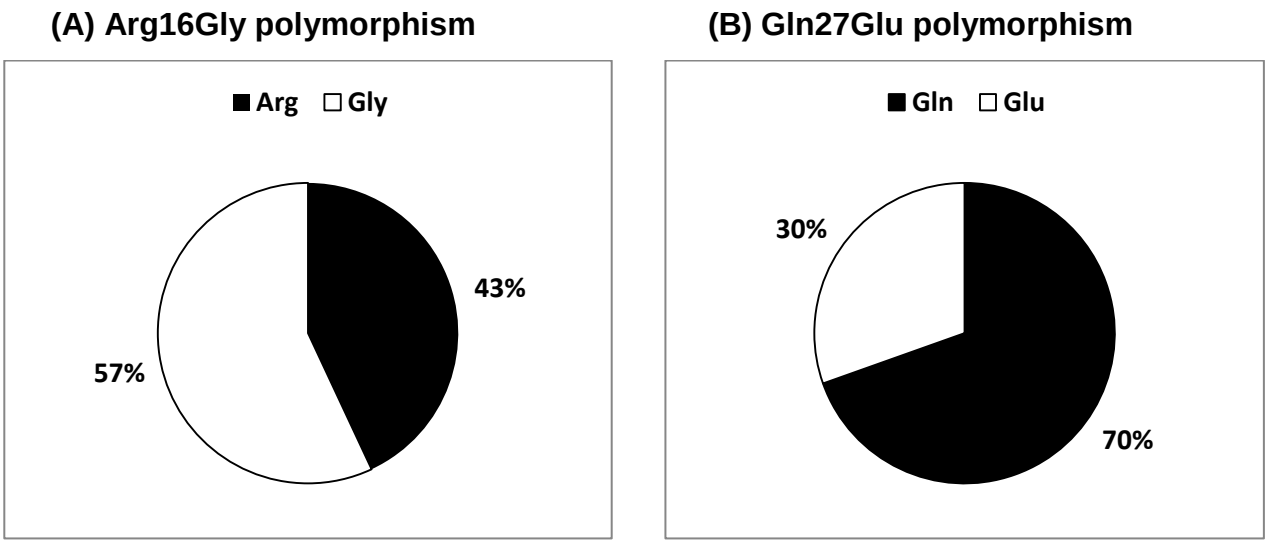


Figure (18). Allele frequencies of *ADRB2* gene polymorphisms among the studied groups (n=115).

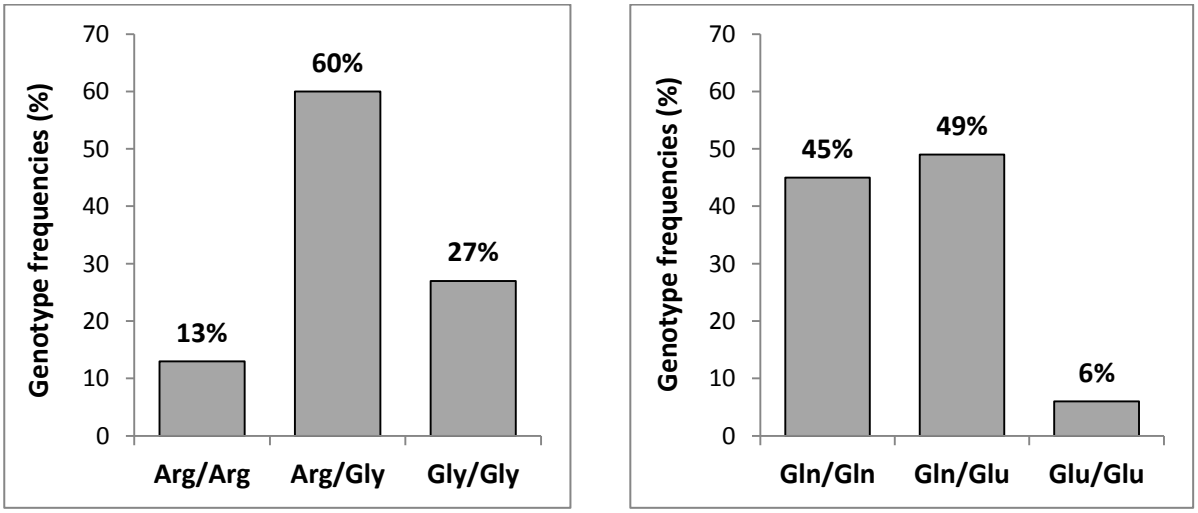


Figure (19). Genotype frequencies of *ADRB2* gene polymorphisms in the study groups (n=115).

Table (17). Genotype frequencies and carriage rates of *ADRB2* gene polymorphisms in COPD patients (n=61) and controls (n=54).

ADRB2 polymorphisms	COPD (n=61)		Controls (n=54)		χ^2	*P* value	OR (95% CI)
Arg16Gly polymorphism							
Genotype frequencies							
Arg_{16}/Arg_{16}	12	(19.7)	3	(5.60)	5.15	0.076	**4.163 (1.107-15.65) ***
Arg_{16}/Gly_{16}	33	(54.1)	36	(66.7)			0.589 (0.276-1.257)
Gly_{16}/Gly_{16}	16	(26.2)	15	(27.7)			0.924 (0.405-2.109)
Carriage rate							
Arg_{16}	45	(73.8)	39	(72.2)	0.034	0.852	1.081 (0.474-2.467)
Gly_{16}	49	(80.3)	51	(94.4)	5.033	**0.025***	**0.240 (0.064-0.903)***
Gln27Glu polymorphism							
Genotype frequencies							
Gln_{27}/Gln_{27}	36	(59.0)	16	(29.6)	13.17	**0.0013***	**3.420 (1.575-7.428) ***
Gln_{27}/Glu_{27}	20	(32.8)	36	(66.7)			**0.244 (0.112-0.531) ***
Glu_{27}/Glu_{27}	5	(8.20)	2	(3.70)			1.825 (0.321-10.38)
Carriage rate							
Gln_{27}	56	(91.8)	52	(96.3)	1.012	0.316	0.430 (0.080-2.317)
Glu_{27}	25	(41.0)	38	(70.4)	9.986	**0.002***	**0.292 (0.135-0.635)***

Values are shown as number (%). COPD, chronic obstructive pulmonary disease. Two-sided Chi square (χ^2) test was used. OR (95% CI), odds ratio and confidence interval; calculated as present *versus* absence. The carriage rate was calculated as the number of individuals carrying at least one of the investigated alleles divided by the total number of individuals in each group. (*) statistically significant at $p<0.05$.

Table (18). Genotype frequencies of *ADRB2* polymorphisms in patients (n=61) and controls (n=54) stratified by smoking status.

Strata	Status	Arg16Gly			*P* value	Gln27Glu			*P* value
		Arg/Arg	Arg/Gly	Gly/Gly		Gln/Gln	Gln/Glu	Glu/Glu	
Smoker	Patients	6	15	6	0.227	17	10	0	0.085
	Controls	1	11	7		7	10	2	
Ex-smoker	Patients	6	17	10	0.714	18	10	5	0.765
	Controls	0	2	1		2	1	0	
Non-smoker	Patients	0	1	0	0.824	1	0	0	0.072
	Controls	2	23	7		7	25	0	

Values are shown as number. Chi square test was used.

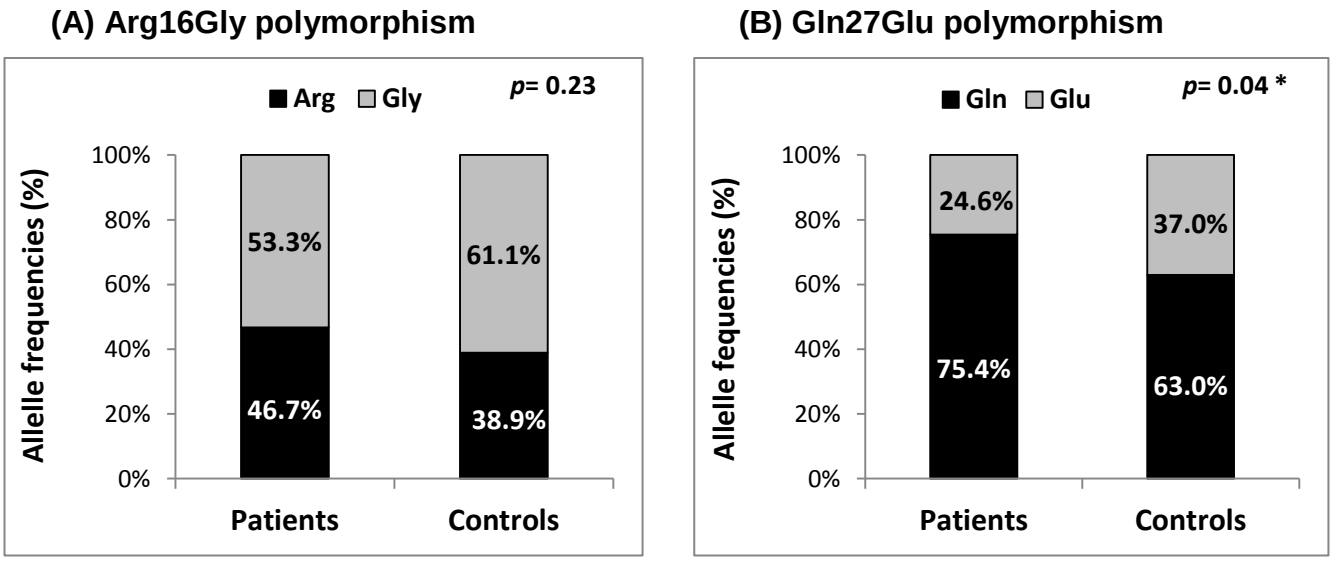


Figure (20). Allele frequencies of *ADRB2* gene polymorphisms in COPD patients (n=61) and controls (n=54). The allele frequency within each group was determined as the number of occurrences of an individual allele divided by the total number of alleles. Two-sided χ^2 test was used. χ^2= 1.43 and 4.19 in Arg16Gly and Gln27Glu polymorphisms, respectively. (*) P-values <0.05 are statistically significant.

Table (19). *ADRB2* gene polymorphisms of the study population in Hardy-Weinberg equilibrium.

Genotype	Observational value		Expectation value		χ^2	P value
COPD patients (n=61)						
Arg16Gly						
Arg_{16}/Arg_{16}	12	(19.7)	13.32	(21.8)	0.23	0.89
Arg_{16}/Gly_{16}	33	(54.1)	30.37	(49.8)		
Gly_{16}/Gly_{16}	16	(26.2)	17.32	(28.4)		
Gln27 Glu						
Gln_{27}/Gln_{27}	36	(59.0)	34.69	(56.9)	0.38	0.82
Gln_{27}/Glu_{27}	20	(32.8)	22.62	(37.1)		
Glu_{27}/Glu_{27}	5	(8.20)	3.69	(6.00)		
Controls (n=54)						
Arg16Gly						
Arg_{16}/Arg_{16}	3	(5.60)	8.17	(15.1)	4.88	0.087
Arg_{16}/Gly_{16}	36	(66.7)	25.67	(47.5)		
Gly_{16}/Gly_{16}	15	(27.7)	20.17	(37.4)		
Gln27 Glu						
Gln_{27}/Gln_{27}	16	(29.6)	21.41	(39.6)	5.80	0.054
Gln_{27}/Glu_{27}	36	(66.7)	25.19	(46.7)		
Glu_{27}/Glu_{27}	2	(3.70)	7.41	(13.7)		

Values are shown as number (%). Hardy-Weinberg calculator: (http://www.oege.org/software/hwe-mr-calc.shtml).

Haplotype analysis

Among the whole study population, only three haplotypes were detected; Arg_{16}/Gln_{27}, Gly_{16}/Gln_{27}, and Gly_{16}/Glu_{27}. Their frequencies were 0.43, 0.27, and 0.3, respectively, Figure (21). Different combinations of haplotypes existed in the study population are shown in Table (20). Whereas, Table (21) demonstrates the effect of carrying each haplotype on disease susceptibility. The carriage rate of Arg_{16}/Gln_{27} haplotype showed no effect on the risk of COPD development (*p*=0.85). However, carrying 2 copies of this particular haplotype increased the risk to develop the disease 4.3 times (*p*=0.023), OR (95%CI) was 4.3 (1.13-16.8). No significant difference between Gy_{16}/Gln_{27} positive and negative individuals (p=0.404), and no influence of copy number of this haplotype on COPD susceptibility (*p*=0.725). However, the carriage rate of Gly_{16}/Glu_{27} haplotype was significantly lower in COPD patients compared to controls (24.3% *versus* 37.3%; *P*= 0.0015), with OR (95% CI) of 0.292 (0.135-0.635). There was no significant difference between individuals heterozygote and homozygote for Gly_{16}/Glu_{27} haplotype (*P*= 0.068), Table (21).

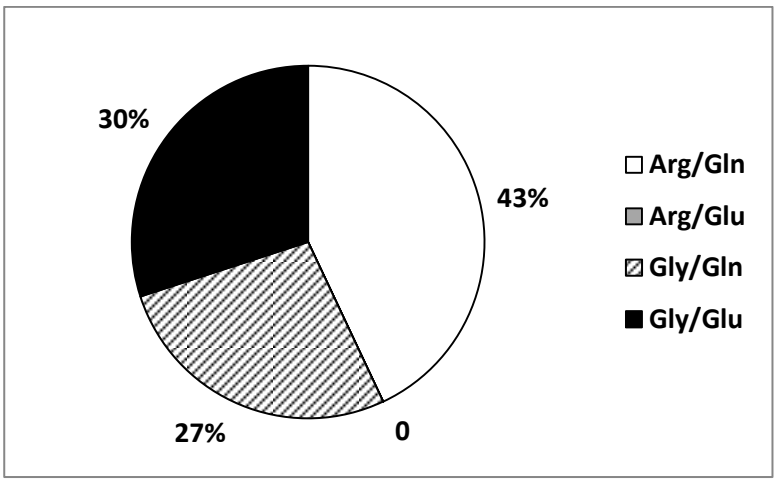


Figure (21). Haplotype frequencies of *ADRB2* gene polymorphisms among the studied groups (n=115).

Table (20). Haplotype combinations of *ADRB2* gene polymorphisms in COPD patients (n=61) and controls (n=54).

Haplotype combinations		COPD		Controls		P value	OR(95%CI)	
Arg_{16}/Gln_{27}	**Arg_{16}/Gln_{27}**	12	(19.7)	3	(5.6)	**0.011***	**4.16**	**(1.10-15.6)***
Arg_{16}/Gln_{27}	**Gly_{16}/Gln_{27}**	22	(36.1)	12	(22.2)		1.46	(0.62-3.41)
Arg_{16}/Gln_{27}	**Gly_{16}/Glu_{27}**	11	(18)	24	(44.4)		**0.24**	**(0.10-0.58)***
Gly_{16}/Glu_{27}	**Gly_{16}/Glu_{27}**	5	(8.2)	2	(3.7)		2.32	(0.43-12.4)
Gly_{16}/Gln_{27}	**Gly_{16}/Glu_{27}**	9	(14.8)	12	(22.2)		0.60	(0.23-1.57)
Gly_{16}/Gln_{27}	**Gly_{16}/Gln_{27}**	2	(3.3)	1	(1.9)		1.79	(0.15-20.3)

Values are shown as number (%).COPD, chronic obstructive pulmonary disease. Two-sided $\chi 2$ test was used (=14.85). OR (95% CI), odds ratio and confidence interval; calculated as present *versus* absence. (*) P-values <0.05 are statistically significant.

Table (21). Haplotype frequencies in COPD patients (n=61) and controls (n=54) according to copy number.

ADRB2 haplotypes	COPD		Controls		χ^2	*P* value	OR (95% CI)
Arg_{16}/Gln_{27} negative	16	(26.2)	15	(27.8)			
Arg_{16}/Gln_{27} positive	45	(73.8)	39	(72.2)	0.034	0.852	1.08 (0.47-2.4)
1 copy	33	(73.3)	36	(92.3)			
2 copies	12	(26.7)	3	(7.7)	5.128	**0.023***	**4.3 (1.13-16.8)***
Gly_{16}/Gln_{27} negative	28	(45.9)	29	(53.7)			
Gly_{16}/Gln_{27} positive	33	(54.1)	25	(46.3)	0.697	0.404	1.36 (0.65-2.8)
1 copy	31	(93.9)	24	(96.0)			
2 copies	2	(6.1)	1	(4.0)	0.12	0.725	1.54 (0.1-18.1)
Gly_{16}/Glu_{27} negative	36	(59.0)	16	(29.6)			
Gly_{16}/Glu_{27} positive	25	(41.0)	38	(70.4)	9.986	**0.002***	**0.29 (0.1-0.63)***
1 copy	20	(80.0)	36	(94.7)			
2 copies	5	(20.0)	2	(5.3)	3.31	0.068	4.5 (0.79-25.3)

Values are shown as number (%). COPD, chronic obstructive pulmonary disease. Two-sided Chi square (χ^2) test was used. OR (95% CI), odds ratio and confidence interval; calculated as present *versus* absence. (*) P-values <0.05 are statistically significant.

ASSOCIATION OF *ADRB2* GENE WITH DISEASE CHARACTERISTICS

Single SNP association analysis

Table (22) demonstrates the association of both polymorphisms with demographic and disease characteristics of COPD patients. The Arg16Gly, but not Gln27Glu polymorphism was associated with disease severity. COPD patients with Arg_{16}/Arg_{16} and Arg_{16}/Gly_{16} displayed more severe degrees of dyspnea, more frequent COPD exacerbations, and higher CAT scores than those with Gly_{16}/Gly_{16} genotype. Arg_{16} allele was significantly associated with more severe grades of dyspnea (Grades III and IV were 40% and 44.4%, respectively), and only 15.6% of patients carrying Arg_{16} allele have dyspnea Grade II; (p=0.011). In contrast, in Gln27Glu polymorphism, patients with Glu_{27} allele was only associated with better CAT scores; (p=0.037), Figures (22). No association was observed between Gln27Glu genotypes and any other patients' clinical characteristics, Table (22).

Haplotype-based association analysis

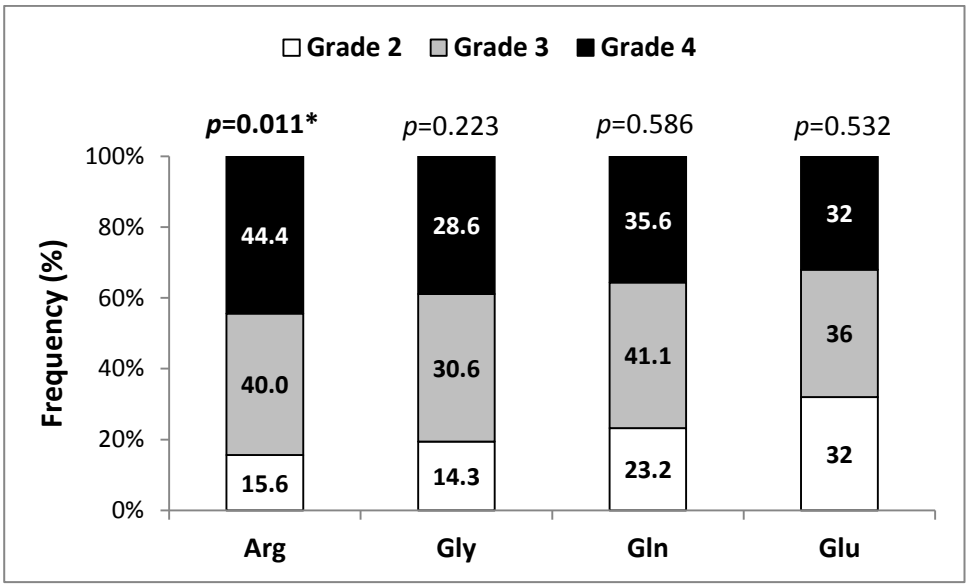
Association of clinical characteristics of COPD patients with *ADRB2* haplotypes is demonstrated in Table (23). The haplotype analysis showed significant association with severity of dyspnea, CAT score, and frequent exacerbations, with higher values among patients with Arg_{16}/Gln_{27} haplotype.

Table (22). Clinical Characteristics of COPD patients according to *ADRB2* genotypes (n=61).

Characteristics	Arg16Gly genotypes			*P value*	Gln27Glu genotypes			*P value*
	Arg/Arg (n=12)	**Arg/Gly** (n=33)	**Gly/Gly** (n=16)		**Gln/Gln** (n=36)	**Gln/Glu** (n=20)	**Glu/Glu** (n=5)	
Age	64 ± 4	62 ± 6	60 ± 7	0.450	62 ± 6	62 ± 7	61 ±0.5	0.527
BMI	23 ± 3	23 ± 5	24 ± 6	0.964	24 ± 4	21 ± 4	28 ±10	0.104
Brinkman index	1343 ±404	1648 ±995	1165 ±341	0.358	1512 ±931	1405 ±624	1195 ±193	0.885
mMRC scale								
Grade 2	1 (8.3)	6 (18.2)	8 (50.0)	**0.023***	2 (5.6)	6 (30)	2 (40)	0.747
Grade 3	7 (58.3)	11 (33.3)	6 (37.5)		15 (41.7)	8 (40)	1 (20)	
Grade 4	4 (33.3)	16 (48.5)	2 (12.5)		14 (38.9)	6 (30)	2 (40)	
CAT score								
Medium	0 (0.0)	16 (50)	13 (81.3)	**0.041***	19 (52.8)	15 (75)	3 (60)	0.107
High	8 (66.7)	9 (37.5)	3 (18.7)		6 (16.7)	4 (20)	2 (40)	
Very high	4 (33.3)	8 (12.5)	0 (0.00)		11 (30.6)	1 (5)	0 (0.0)	
Exacerbations								
<2 times	3 (25)	9 (27.3)	11 (68.7)	**0.012***	14 (38.9)	6 (30)	3 (60)	0.453
≥2 times	9 (75)	24 (72.7)	5 (31.3)		22 (61.1)	14 (70)	2 (40)	
ICU admission								
No	10 (83.3)	23 (69.7)	11 (68.7)	0.626	25 (69.4)	16 (80)	3 (60)	0.574
Yes	2 (16.7)	10 (30.3)	5 (31.3)		11 (30.6)	4 (20)	2 (40)	

Values are shown as mean ± standard deviation or number (%). BMI, body mass index; Brinkman smoking index, number of cigarettes per day times the number of years; mMRC scale, (Modified Medical Research Council) scale for dyspnea severity; CAT, COPD Assessment Test for estimating impact level of COPD on health status; ICU, intensive care unit. Two-sided Chi-square test and Kruskal-Wallis were used. (*) P-values <0.05 are statistically significant.

(A) MMRC scale



(B) CAT score

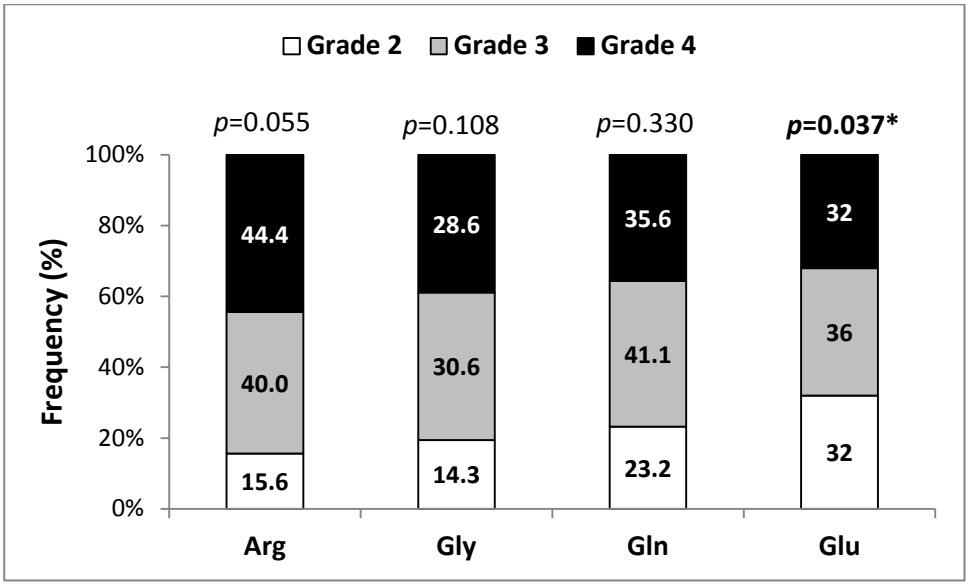


Figure (22). MMRC scale and CAT scores in COPD patients according to *ADRB2* alleles (n=61). Two-sided χ^2 test was used.

Table (23). Clinical Characteristics of COPD patients according to *ADRB2* haplotypes (n=61).

Characteristics	Arg/Gln		*P value*	Gly/Gln		*P value*	Gly/Glu		*P value*
	Positive (n=45)	Negative (n=16)		Positive (n=33)	Negative (n=28)		Positive (n=25)	Negative (n=36)	
Demographic data									
Age	62.6 ± 5.6	60.1 ± 6.9	0.152	61.1 ± 7.4	63.0 ± 3.6	0.211	61.5 ± 6.3	62.3 ± 5.8	0.590
BMI	22.8 ± 3.9	23.7 ± 6.0	0.532	23.7 ± 4.2	22.3 ± 4.8	0.215	21.8 ± 5.3	23.9 ± 3.7	0.079
Brinkman index	1165 ± 342	1564 ± 880	0.171	1423 ± 999	1369 ± 558	0.801	1239 ± 606	1512 ± 931	0.205
Disease severity									
MMRC scale	3.2 ± 0.7	2.6 ± 0.7	**0.003***	3.0 ± 0.8	3.2 ± 0.7	0.221	3.0 ± 0.8	3.2 ± 0.7	0.340
CAT score	24.8 ± 8.1	19.1 ± 6.2	**0.013***	23.1 ± 8.0	23.5 ± 8.0	0.820	24.3 ± 8.6	21.9 ± 6.9	0.255
Exacerbations	2.0 ± 0.8	1.7 ± 1.6	**<0.01***	2.0 ± 1.3	1.9 ± 0.8	0.091	2.0 ± 1.3	1.9 ± 0.8	0.913
ICU admission	0.2 ± 0.4	0.3 ± 0.4	0.725	0.3 ± 0. 4	0.2 ± 0.4	0.108	0.2 ± 0.4	0.3 ± 0.4	0.574

Values are shown as mean ± SD. Mann-Whitney U test was used.

ASSOCIATION OF *ADRB2* GENE WITH BBRONCHODILATOR RESPONSE

Single SNP association analysis

The COPD patients were further subdivided according to their FEV_1 values in agreement with American Thoracic Society (ATS) staging system for the severity of CODP (GOLD stage). No statistical difference was found between patient groups according to their genotypes, Figure (23). The genotypic frequencies containing Gly_{16} and Gln_{27} alleles showed more severe airflow obstruction with FEV_1 values <35% of predicted, however, they did not reach significant difference (p=0.12 and p=0.247 for Arg16Gly and Gln27Glu polymorphisms, respectively).

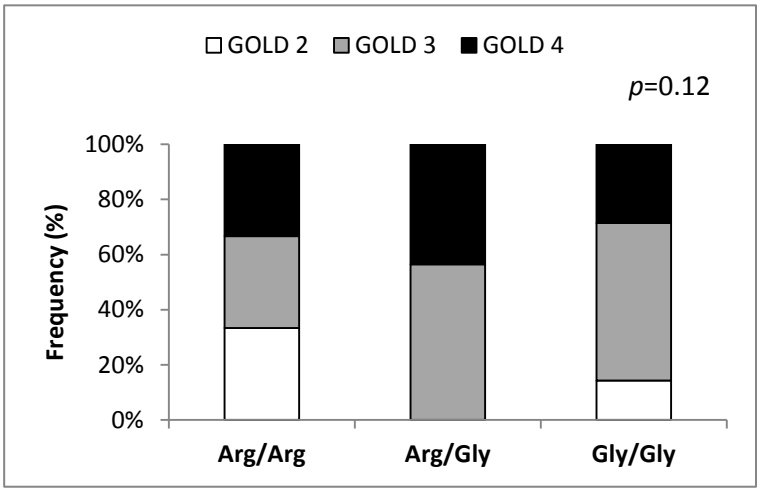
Association of baseline spirometric characteristics of COPD patients and bronchodilator response after salbutamol with *ADRB2* genotypes are summarized in Table (24). No association was observed between Arg16Gly polymorphism with BDR. In contrast, Gln27Glu polymorphism was associated with BDR, with higher values in patients with Glu_{27}/Glu_{27} genotype compared to Gln_{27}/Gln_{27} and Gln_{27}/Glu_{27} genotypes (p=0.022), Figure (24). Similarly, COPD patients carrying Glu_{27} allele and those not carrying Gln_{27} had better response (p=0.032 and 0.012, respectively), Figure (25).

Haplotype-based association analysis

Table (25) shows correlation between different *ADRB2* haplotypes and pulmonary function parameters. The haplotype analysis showed that Arg_{16}/Gln_{27} haplotype had no association with BDR. However, individuals who are carrying Gly_{16}/Gln_{27} haplotype had worse BDR than their counterparts (4.2±3.6 *versus* 7.5±4.2; p=0.016). Whereas, COPD patients with Gly_{16}/Glu_{27} had better response than those not carrying it (7.0±4.5 *versus* 4.2±3.4; p=0.032), Figure (26). The copy numbers of Gly_{16}/Glu_{27} haplotype did not show dose-response relationship on comparing homozygote patients for

Gly_{16}/Glu_{27} haplotype with heterozygote patients having Gly_{16}/Glu_{27} + Gly_{16}/Gln_{27} or Gly_{16}/Glu_{27} + Arg_{16}/Glu_{27} haplotypes (p=0.101).

(A) Arg16Gly polymorphism



(B) Gln27Glu polymorphism

Figure (23). GOLD stage in COPD patients according to *ADRB2* genotype (n=43). Two-sided Chi-square test was used.

Table (24). Pulmonary function parameters of COPD patients before and after salbutamol according to *ADRB2* genotypes (n=43).

Characteristics	Arg16Gly genotypes			*P* value	Gln27Glu genotypes			*P* value
	Arg/Arg (n=6)	Arg/Gly (n=23)	Gly/Gly (n=14)		Gln/Gln (n=25)	Gln/Glu (n=14)	Glu/Glu (n=4)	
Pre-bronchodilator								
FEV_1 (% predicted)	38.4±18.2	30.2±11.8	34.8±12.2	0.624	30.6±13.9	36.9±13.7	32.8±1.2	0.309
FVC (% predicted)	54.9±19.8	47.1±15.5	48.4±17.0	0.847	46.9±16.0	50.3±19.6	52.9±1.0	0.539
FEV_1/FVC	53.3±6.2	50.1±7.2	58.3±7.1	**0.006***	50.5±7.4	59.4±5.9	48.8±1.4	**0.001***
Post-bronchodilator								
FEV_1 (% predicted)	40.3±18.9	31.9±12.9	36.9±12.5	0.470	31.9±13.9	39.5±14.7	35.9±0.4	0.196
FEV_1/FVC	53.8±7.6	50.0±7.9	59.3±6.5	**0.006***	50.2±8.2	60.4±4.8	50.9±4.5	**0.001***
BDR								
BDRABS (mL)	60.0±38.9	45.7±49.3	57.9±41.5	0.666	36.8±35.3	65.7±53.5	95.0±28.9	0.075
BDRPRED (%)	1.9±1.3	1.6±1.8	2.1±1.5	0.696	1.3±1.3	2.5±2.0	3.1±0.8	0.192
BDRBASE (%)	5.6±4.2	4.8±4.1	6.3±4.2	0.551	4.2±3.4	6.3±4.7	9.6±2.7	**0.022***

Values are shown as mean ± SD. Kruskal-Wallis test was used.

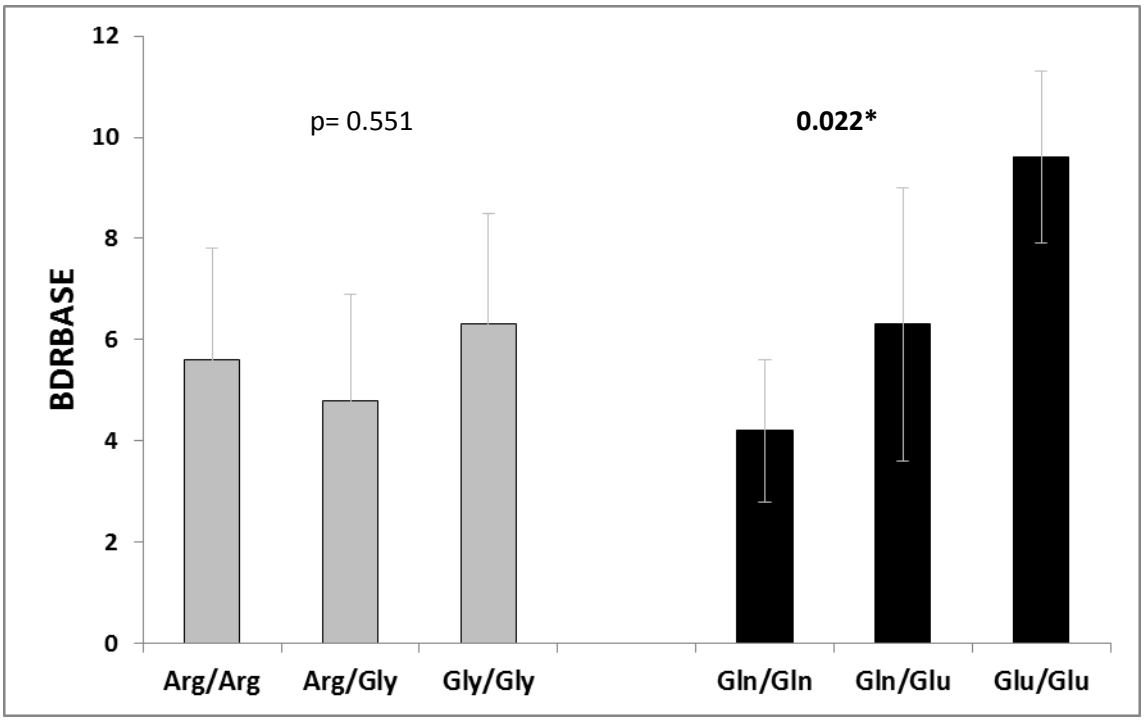


Figure (24). Bronchodilator response in COPD patients according to *ADRB2* genotypes (n=43). Kruskal-Wallis test was used.

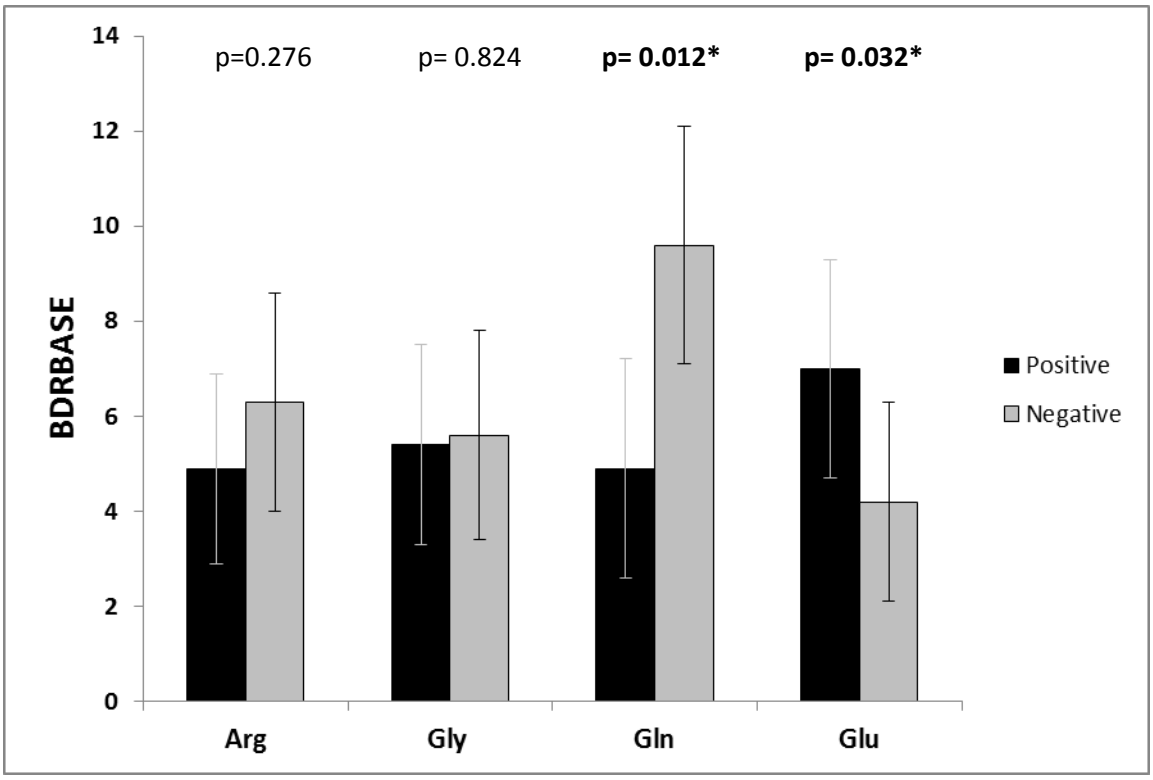


Figure (25). Bronchodilator response in COPD patients according to *ADRB2* alleles (n=43). Mann-Whitney U test was used.

Table (25). Pulmonary function parameters of COPD patients before and after salbutamol according to *ADRB2* haplotypes (n=43).

Characteristics	Arg/Gln		*P* value	Gly/Gln		*P* value	Gly/Glu		*P* value
	Positive (n=29)	Negative (n=14)		Positive (n=27)	Negative (n=16)		Positive (n=18)	Negative (n=25)	
Pre-bronchodilator									
FEV_1 (% predicted)	31.9 ±13.4	34.8 ±12.2	0.622	31.4 ±12.7	35.3 ±13.5	0.580	36.0 ±12.1	30.6 ±13.3	0.192
FVC (% predicted)	48.7 ±16.4	48.4 ±17.0	0.938	46.5 ±16.1	52.2 ±16.9	0.365	50.9 ±17.1	46.9 ±16.0	0.268
FEV_1/FVC	50.8 ±7.0	58.3 ±7.0	**0.002**	53.5 ±9.0	52.7 ±5.4	0.801	57.0 ±6.9	50.5 ±7.4	**0.005**
Post-bronchodilator									
FEV1 (% predicted)	33.6 ±14.4	36.9 ±12.5	0.421	32.7 ±13.2	38.0 ±14.6	0.291	38.7 ±12.9	31.9 ±13.9	0.08
FEV1/FVC	66.9 ±10.5	77.5 ± 8.2	**0.002**	69.9 ±12.6	71.2 ±7.5	0.801	76.4 ±7.6	66.0 ±10.9	**0.002**
BDR									
BDRABS (mL)	48.6 ±47.1	57.9 ±41.5	0.505	34.8 ±34.7	80.0 ±47.3	**0.008**	72.2 ±49.8	36.8 ±35.3	0.054
BDRPRED (%)	1.7 ±1.7	2.1 ±1.6	0.622	1.3 ±1.4	2.7 ±1.7	**0.016**	2.6 ±1.8	1.3 ±1.3	0.132
BDRBASE (%)	4.9 ±4.1	6.3 ±4.2	0.276	4.2 ±3.6	7.5 ±4.2	**0.016**	7.0 ±4.5	4.2 ±3.4	**0.032**

Values are shown as mean ± SD. Mann-Whitney U test was used.

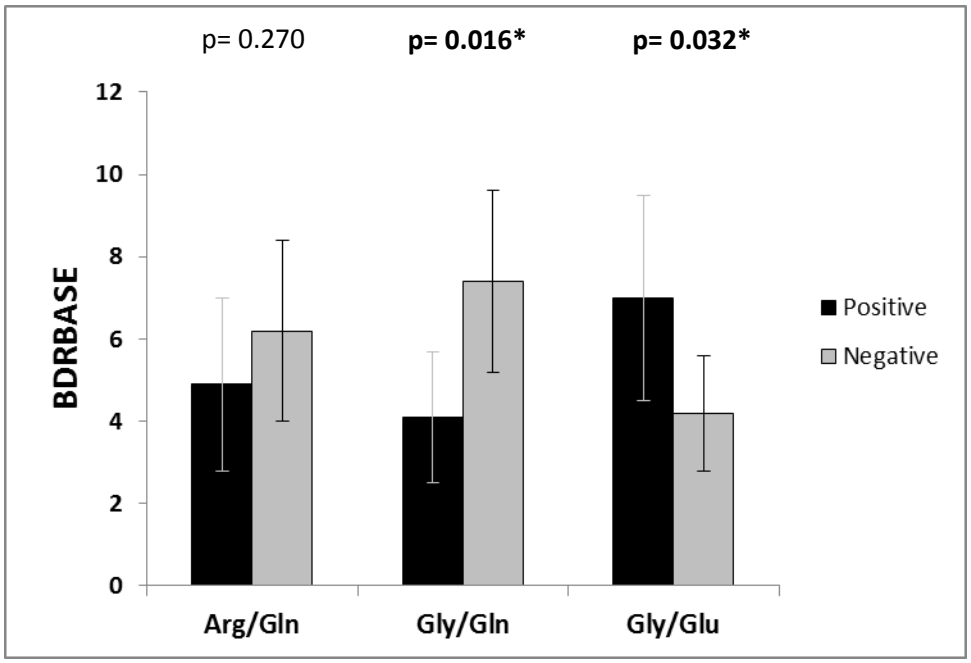


Figure (26). Bronchodilator response in COPD patients according to *ADRB2* haplotypes (n=43). Mann-Whitney U test was used.

Discussion

Chronic Obstructive Pulmonary Disease (COPD) is a major cause of chronic morbidity and mortality. The World Health Organization (WHO) listed COPD as the fourth cause of death in the world and it is expected to be the third cause of death globally by 2020 (***Papatheodorou et al., 2010***). Beta$_2$-adrenergic agonists are the most widely bronchodilators used to treat airflow limitations associated with COPD. Inter-individual variation in therapeutic responses to β_2-agonists was reported among COPD patients in several studies (***Hizawa et al., 2007; Cho, 2010; Qiu et al,. 2010***).

The β_2-adrenergic receptor (ADRB2) is a trans-membrane protein expressed on the surface of airway smooth muscle cells that play a key role in tuning airway function (***Mokry et al., 2008; Karam et al., 2013***). Several coding polymorphisms of the *ADRB2* gene have been found to alter receptor function. The polymorphisms at amino acid position 16 (Arg16Gly) and position 27 (Gln27Glu) are particularly prevalent, functionally relevant, and have been extensively studied from the clinical and pharmacological perspectives (***Karam et al., 2013; Paiva et al., 2014***).

Recent updates on COPD guidelines recommended that all patients should be screened for their genetic subtypes, which may affect treatment decisions (***Yawn et al., 2012***). This study was conducted to assess the genetic association between *ADRB2* gene polymorphisms and COPD among Egyptian patients, to unravel the potential importance of the *ADRB2* genotype as a marker for COPD susceptibility and to identify the polymorphism which may influence disease prognosis and treatment decision in Egyptian patients.

To date, only one study has examined the association of genetic variation in *ADRB2* with risk of COPD development among Egyptian patients (***Hegab et al., 2004***), but – up to our knowledge - no other studies looked at the possible role of

ADRB2 haplotypes on the clinical outcome or bronchodilator response in Egyptian COPD patients has been published.

Among the study groups, Gly_{16} and Gln_{27} were the most prominent alleles, representing 57% and 70% of the population, respectively. Correspondingly, Arg_{16}/Gly_{16} and Gly_{16}/Gly_{16} at codon 16 and Gln_{27}/Gln_{27} and Gln_{27}/Glu_{27} at codon 27 were the most frequent genotypes. This was consistent with the study conducted by ***Hegab*** et al. (***2004***), who reported similar results in Egyptian population; Gly_{16} and Gln_{27} were the most frequent alleles among their study groups representing 60.1% and 75.8%, respectively. ***Abu-Amero*** et al. (***2006***) also showed that the homozygous Gln_{27}/Gln_{27} genotype was the most predominant, while the Glu_{27}/Glu_{27} was the least prevalent in the Saudi population. Similarly, studies of ***Contopoulos-Ioannidis*** et al. (***2005***), ***Augusto*** et al. (***2010***), and ***Chung*** et al. (***2011***) demonstrated the higher frequencies of Gly_{16} and Gln_{27} polymorphisms in healthy Caucasians and African-Americans. In a different population, ***Xie*** et al. (***1999***) and ***Augusto*** et al. (***2010***) showed Arg_{16} allele to be more prevalent among Chinese population than Gly_{16} allele. Also, ***Hegab*** et al. (***2004***), ***Contopoulos-Ioannidis*** et al. (***2005***), and ***Limsuwan*** et al. (***2010***) reported higher frequency of Gln_{27} polymorphism and absence of Glu_{27}/Glu_{27} genotype in individuals with Asian descent (Japanese, Chinese, and Thai populations). These results confirm the ethnic variability in allele frequencies of *ADRB2* reported in several studies (***Lucas et al., 2004; Jalba et al., 2008; Chung et al., 2011***).

<u>Haplotype analysis</u> among our study population revealed the presence of 3 haplotypes only. Their frequencies were 43% for Arg_{16}/Gln_{27}, 27% for Gly_{16}/Gln_{27}, and 30% for Gly_{16}/Glu_{27}. The absence of Arg_{16}/Glu_{27} in our study population was consistent across several haplotype studies conducted in different ethnic populations, including African-Americans, Asians and Hispanic-Latinos populations (***Drysdale et al., 2000; Israel et al., 2004, Hawkins et al., 2006; Hesse and Eisenach, 2008; Limsuwan et al., 2010***). Similarly, ***D'Ameto*** et al.

(***1998***), ***Vacca*** et al. (***2009***), and ***Paiva*** et al. (***2014***) reported the absence of this variant among Italian, German, and Brazilian populations, respectively. Other studies revealed the Arg_{16}/Glu_{27} haplotype to be an extremely rare with frequency <1% in Caucasian populations (***Martinez et al., 1997; Chung et al. 2011***). This absence was explained by the studies of ***Hegab*** et al. (***2004***), ***Contopoulos-Ioannidis*** et al. (***2005***), ***Limsuwan*** et al. (***2010***), and ***Chung*** et al. (***2011***) that revealed tight linkage disequilibrium (LD) between the two polymorphisms within the *ADRB2* gene, and as a result, the Arg_{16} allele is typically seen with Gln_{27}, whereas Gly_{16} can coexist with either Gln_{27} or Glu_{27}.

ADRB2* polymorphisms *among COPD patients and healthy controls, in the current study, demonstrated that all alleles and genotype frequencies among COPD patients and healthy individuals were in accordance with the Hardy-Weinberg Equilibrium. Regarding Arg16Gly polymorphism, Arg_{16}/Arg_{16} genotype was 4.1 times more likely than Arg_{16}/Gly_{16} and Gly_{16}/Gly_{16} to develop COPD. This risk existed only if the individual had 2 copies of Arg_{16} allele. In contrast, the Gly_{16} allele was associated with low risk of COPD. This was in agreement with studies by ***Matheson*** et al. (***2006***) and ***Yang*** et al. (***2007***) who showed that Arg_{16} homozygotes had increased risk of obstructive airway diseases (asthma and COPD) in Caucasian patients. However, this was inconsistent with other studies by ***Ho*** et al. (***2001***) and ***Vacca*** et al. (***2009***) who reported an association of Gly_{16} with more susceptibility to COPD development than healthy individuals and lower frequency of the homozygote Arg_{16}/Arg_{16} genotype among Chinese and German COPD patients. On the other hand, ***Brøgger*** et al. (***2006***) and ***Papatheodoroua*** et al. (***2010***) found no significant differences in genotype frequencies among Nordic and Greek populations. Another study conducted upon Chinese COPD patients by ***Niu*** et al. (***2012***), also revealed that *ADRB2* polymorphism at position 16 have no effect on the occurrence of COPD.

For Gln27Glu polymorphism, genetic assessment in our study revealed a significant association with the development of COPD in Egyptian population.

The frequency of Gln_{27} allele was more predominant in COPD patients compared to that of the control group. On the other hand, Glu_{27} allele was more frequent in healthy controls. Correspondingly, Gln_{27}/Gln_{27} was significantly the most predominant genotype in COPD patients; those carrying this genotype were 3.4 more likely to develop COPD than their counterparts. While in the healthy control group, the heterozygote Gln_{27}/Glu_{27} genotype was the most prevalent genotype. Similarly, ***Joos*** et al. (***2003***), ***Tang*** et al. (***2007***), ***Lee*** et al. (***2009***), and ***Marson*** et al. (***2012***) reported that the presence of Glu_{27} in heterozygote individuals (Gln_{27}/Glu_{27}) was protective against obstructive pulmonary disease. Also, ***Hopes*** et al. (***1998***) and ***Matheson*** et al. (***2006***) showed that the Gln_{27} homozygotes had an increased risk of asthma and bronchial hyper-responsiveness. In contrast, ***Hegab*** et al. (***2004***) reported a higher frequency of Glu_{27} allele among COPD patients. On the other hand, in several other studies conducted by ***Ho*** et al. (***2001***), ***Molfino*** (***2004***), ***Vacca*** et al. (***2009***), and ***Niu*** et al (***2012***), no significant role of the Gln27Glu polymorphism was found in COPD development.

The difference between our result and some prior studies could be caused by the genetic heterogeneity among different ethnic groups (***Drysdal et al., 2000***). Since the susceptibility to COPD is affected by multiple genetic factors and genotype-environment interactions (multifactorial inheritance mode), in which each factor has a small influence on the development of the disease (***Slabá et al., 2008; El-Zein, 2012; Paive et al., 2014***). Another possibility is that some polymorphisms can be responsible for COPD in one ethnic group and not in others (***Hegab et al., 2004; Vercelli, 2008***). Another explanation for the differences is that COPD is a heterogeneous disorder, characterized by dysfunction of airways and destruction of the lung parenchyma, both of which can lead to the progressive airflow limitation (***Marson et al., 2012***). Therefore, it could be suggested that patients with COPD in different ethnic groups have distinct type of genotype causing a specific aspect of the COPD spectrum: chronic bronchitis and/or emphysema (***Hegab et al., 2004; Slabá et al., 2008***).

Thus variations in the frequency of SNPs in different populations, the modulating effects of other SNPs or mutations within individuals, variation in the penetrance of a SNP due to other factors as age-effect, different phenotypic spectrum of the disease, different gender of enrolled study populations, and the difficulty in matching for all known environmental factors that predispose to COPD could provide other plausible explanations for the conflicting results between studies.

On comparing different haplotype combinations between our COPD patients and healthy volunteers, there was a significant difference in their haplotype frequencies. Homozygotes for Arg_{16}/Gln_{27} haplotype were associated with more than 4 times increased risk of developing COPD. But Arg_{16}/Gln_{27} individuals, heterozygote with other haplotype combination (Arg_{16}/Gln_{27} + Gly_{16}/Gln_{27} or Arg_{16}/Gln_{27} + Gly_{16}/Glu_{27}), did not show any association with disease susceptibility, thus highlighting a clear dose-response relationship. On the other hand, the carriage rate of Gly_{16}/Glu_{27} haplotype was protective; with lower frequency in COPD patients. No significant difference was found in homozygote and heterozygote individuals for that haplotype, therefore eliminating the potential gene-dosage effect of Glu_{27} allele for the occurrence of COPD disease. However, Gly_{16}/Gln_{27} haplotype did not influence the susceptibility for developing COPD in our Egyptian population. This was consistent with two clinical studies by ***Matheson*** et al. (***2006***) and ***Hizawa*** et al. (***2007***) who revealed that Arg_{16}/Gln_{27} haplotype was associated with increased risk of COPD in Australian and Japanese populations.

Several mechanisms could explain the association between Arg_{16}/Gln_{27} haplotype homozygosity and COPD development; ***Thakkinstian*** et al. (***2005***) demonstrated the altered activity of β_2-adrenoceptors by the over-dominant protective effect of Glu_{27} allele in heterozygote haplotypes. And as mentioned before, ***Limsuwan*** et al. (***2010***) along with others revealed that Glu_{27} cannot coexist with Arg_{16} allele due to linkage disequilibrium. Thus the absence of Glu_{27} allele may be the real factor underlying the increased risk of COPD. However,

there is an alternative possibility is that since ***Drysdal*** et al. (***2000***) described other molecular haplotypic structure of *ADRB2* including our 2 SNPs, Arg16Gly and Gln27Glu polymorphisms might be in linkage disequilibrium with another SNP in *ADRB2* gene or nearby inflammatory mediator genes that have direct effect on the pathogenesis of COPD (***Ge et al., 2005***). In addition, several lines of evidence indicated that *ADRB2*, like other G protein coupled receptors, form dimers. The formation of β_2-AR dimers was shown to have functional effects on receptor stimulated adenylate cyclase activity (***Hebert et al., 1996***). So one possible mechanism is the interaction between 2 receptors with Arg_{16}/Gln_{27} haplotype forming 'homodimer' may have different agonist binding, signal transduction, or agonist-promoted desensitization properties than other types of dimers.

Concerning the association between ADRB2 polymorphisms and disease severity among our COPD patients, single SNP association analysis revealed that individuals with Arg_{16} allele and genotypes had more severe grades of dyspnea according to the Modified Medical Research Council (mMRC) scale for dyspnea grading and higher impact of COPD disease on health status as represented by COPD Assessment Test (CAT) score for symptom assessment. Also patients with Arg_{16}/Arg_{16} and Arg_{16}/Gly_{16} displayed more frequent COPD exacerbations. Similarly, ***Taylor*** et al (***2000***) and ***Larocca*** et al. (***2012***) also reported higher frequency of asthma exacerbations among patients with Arg_{16} allele. But we did not find any previous published studies testing association between Arg16Gly polymorphism and COPD severity. However, genetic analysis at position 27 among our patients revealed that the Glu_{27} allele was significantly associated with better CAT scores and low impact of COPD disease on health status. This was in line with studies of ***Green*** et al. (***1994*** and ***1995***), ***Ho*** et al. (***2001***), and ***Papatheodoroua*** et al. (***2010***) who showed that Gln_{27} allele was associated with more severe airflow obstruction in COPD patients. In addition, studies of ***McGraw*** et al. (***2003***) and ***Shore and Drazen*** (***2003***) revealed that genetic variations of *ADRB2* influence the activation of extracellular signal-regulated

kinases in airway smooth muscle cells and contribute to exaggerated inflammatory cytokine expression, thus could provide potential mechanisms underlying the genetic impact of *ADRB2* polymorphisms on disease severity and frequent exacerbations.

Haplotype-based analysis in our cases also showed significant association of *ADRB2* gene polymorphisms with the severity of dyspnea and CAT score. Patients with Arg_{16}/Gln_{27} haplotype displayed more severe symptoms of COPD, with no significant difference between homozygote and heterozygote patients carrying this haplotype. This was consistent with ***Matheson*** et al. (***2006***) who reported that Arg_{16}/Gln_{27} haplotype may be associated with more severe respiratory symptoms in middle-aged and older adults. This could be explained by ***Synder*** et al. (***2008***) who found Arg_{16} allele to represent a condition with less ability to clear fluid from the lungs compared to the Gly_{16} variant, due to a decrease in alveolar-capillary membrane conductance in this group. ***Lai and Rogers*** (***2010***) ***and Oldenburger*** et al. (***2012***) demonstrated that both mucus hyper-secretion and ciliary dysfunction are typically the first physiological abnormalities in COPD. ***Rogers*** (***2001***) and ***Hurst*** et al. (***2010***) also reported that mucus hyper-secretion contributed to morbidity and mortality in certain groups of patients with COPD. Collectively, previous studies of ***Dishy*** et al. (***2001***), ***Garovic*** et al. (***2003***), and ***Eisenach*** et al. (***2004***) have suggested that the Arg_{16} group has reduced receptor function when compared to the Gly_{16} group *in vivo*. These observations may provide plausible explanation for the association of genotypes and haplotypes containing Arg_{16} allele with more severe disease.

Baseline pulmonary function of our COPD patients, in our study, showed no differences in FEV_1 among patients with various *ADRB2* genotypes at position 16 and 27. This was consistent with two previous clinical studies conducted by ***Brøgger*** et al. (***2006***) and ***Niu*** et al. (***2012***) who reported the absence of association between Arg16Gly and Gln27Glu genotypes and FEV_1 in Nordic and Chinese COPD patients. A study in Denmark by ***Thomsen*** et al.

(***2012***) also reported no association with lung function in COPD. Similarly, ***Joos*** et al. (***2003***) showed no effect on baseline pulmonary function in smokers with mild-to-moderate COPD. Also ***Martinez*** et al. (***1997***) demonstrated no difference of *ADRB2* genotypes on FEV_1 and FVC in children. In contrast, studies by ***Ho*** et al. (***2001***) and ***Papatheodorou*** et al. (***2010***) demonstrated lower FEV_1 values in COPD patients with Gly_{16} and Gln_{27} polymorphism among Chinese and Greek populations. Haplotype analysis of *ADRB2* gene polymorphisms among our patients was in agreement with studies of ***Woszczek*** et al. (***2005***) and ***Brøgger*** et al. (***2006***) who showed no effect of *ADRB2* haplotypes on FEV_1 among European Caucasians (Polish and Nordic populations). However, this was in contrast to a study conducted in Slovakia by ***Mokry*** et al. (***2008***) who reported lower FEV_1 in COPD patients carrying Gly_{16}/Glu_{27} haplotype. This difference in *ADRB2* association with lung function phenotypes between ethnic groups was reported previously by ***Hawkins*** et al. (***2006***).

On examining bronchodilator response (BDR) after salbutamol inhalation among our COPD patients, we found no effect on Arg16Gly polymorphism. This was consistent with ***Joos*** et al. (***2003***) who reported no association of Arg16Gly polymorphism with BDR. This was also in agreement with ***Leineweber*** et al. (***2010***) who reported that Arg16Gly polymorphism did not affect β_2-AR function (ligand binding, or adenylate cyclase activity) in hamster fibroblasts. In addition, ***Small*** et al. (***2003***) reported that receptor synthesis rates and agonist-promoted internalization were not different between the receptors. However, other investigators reported contradictory results; ***Silverman*** et al. (***2003***), ***Woszczek*** et al. (***2005***) and ***Qiu*** et al. (***2010***) showed higher BDR in patients with Arg_{16}/Arg_{16} homozygotic genotype in comparison with respective homo- and heterozygotes. On contrary, ***Hizawa*** et al. (***2007***) reported the reverse; they demonstrated that the presence of the Arg_{16} allele in Japanese patients with stable COPD was associated with a lower BDR to β_2-agonist inhalation.

As for Gln27Glu polymorphism, our results showed an interaction between Gln27Glu genotypes and BDR to salbutamol, with patients with Gln_{27}/Gln_{27} exhibiting the smallest response, patients with Gln_{27}/Glu_{27} an intermediate response, and patients with Glu_{27}/Glu_{27} the best response. Similarly, patients with Glu_{27} allele and those without Gln_{27} allele had significantly better BDR to salbutamol compared to their counterparts. Similarly, ***Cockcroft*** et al. (***2000***) and ***Synder*** et al. (***2006***) suggested that the Gln_{27} polymorphism has attenuated receptor function in response to the administration of a β_2-agonist in healthy subjects. ***Dishy*** et al. (***2001***) and ***Litonjua*** et al. (***2010***) also reported that individuals who were homozygous for Glu_{27} had higher maximal bronchial dilatation in response to β_2-agonists than those who were homozygous for Gln_{27}, suggesting that the Gln to Glu change is associated with increased agonist-mediated responsiveness. This was consistent with a previous study by ***Johnson*** et al. (***2006***) who showed the *ADRB2* polymorphisms would alter the functional properties of the receptor such that the airways of individuals react differently when exposed to circulating catecholamines or exogenously applied β_2-agonists. This difference in response may be due to alterations in receptor degradation after the internalization step (***Small et al., 2003; Bernardez et al., 2010; Masuo and Lambert, 2011; Adriani et al., 2012***).

Haplotype-based analysis in the present study showed better response in patients carrying Gly_{16}/Glu_{27} and those not carrying Gly_{16}/Gln_{27} haplotypes, with no dose-response relationship according to their copy number in the individual. This was consistent with an *in vivo* study by ***Drysdale*** et al. (***2000***) and ***Limsuwan*** et al. (***2010***) indicating that the Gly_{16}/Glu_{27} haplotype leads to an improved response to bronchodilators. Similar results were shown in two further clinical studies by ***Cho*** (***2010***) and ***Qiu*** et al. (***2010***) who reported that the patients with the homozygous Gly_{16}/Gln_{27} haplotype pairs showed the lowest BDR, while patients with the homozygous Arg_{16}/Gln_{27} haplotype pairs and those heterozygous for Arg_{16}/Gln_{27} + Gly_{16}/Glu_{27} combination showed the highest

response. This may be because the Glu_{27} form of the receptor was known to protect against down-regulation (***Johnson et al., 2006; Limsuwan et al., 2010; Karam et al., 2013***). *In vitro* studies by ***Green*** et al. (***1993 and 1995***) and ***Tan*** et al. (***1997***) showed that primary human airway smooth muscle cells has down-regulated Glu_{27} isoform to a much lesser extent than the Gln_{27} receptor after prolonged exposure to β_2-agonists. In addition, a similar relative resistance to down-regulation was observed by ***Holroyd*** et al. (***1995***) using β_2-agonist–mediated cAMP formation as an end point for receptor coupling. A following study by ***Iaccarino*** et al. (***2006***) confirmed the resistance of the Glu_{27} receptor variant to agonist-promoted down-regulation by performing cell-signaling studies in two cell lines rather than measuring cAMP; the Glu_{27} variant magnified the catecholamine-induced activation of intracellular signaling transduction. Together these results indicate a protective role of Glu_{27} in respiratory airway.

In summary, our results demonstrated that the Arg16Gly and Gln27Glu polymorphisms of the *ADRB2* gene, individually and in combination, play an important role in COPD. Homozygotes for Arg_{16} or Gln_{27} and Arg_{16}/Gln_{27} haplotype had an increased risk for developing COPD. In addition, genotypes and haplotypes containing Arg_{16} allele were associated with more severe COPD symptoms. However, individuals with Gln_{27} polymorphism were associated with lower BDR in Egyptian population. The Glu_{27} allele and haplotypes conferred protection against disease prevalence and severity, and improved bronchial airway responsiveness to β_2-agonists.

Overall, our study had few limitations: our sample size may be considered small, there was no control for environmental factors, and only two polymorphisms were analyzed. In addition, the Egyptian population is admixed; and a region with a specific genotype combination associated with risk may also be associated with a peculiar environmental factor.

Further efforts at replication of significant genetic associations using large

sample sizes and demonstration of functional significance of associated genetic variants using animal models and/or *in vitro* systems will be required to confirm the pathogenic role of *ADRB2* as genetic determinants of COPD susceptibility and prognosis. Further studies are recommended to understand the possible role of these genetic variations in regulating responses to COPD therapy in the Egyptian population.

Summary

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide, and represents a huge and growing economic and social burden (***Berndt et al., 2012; Zhang et al., 2014***). It is characterized by chronic inflammation and irreversible airflow obstruction, involving structural changes in the lung (***Brashier and Kodgule, 2012***).

COPD is a multi-factorial disorder caused by environmental determinants and genetic risk factors (***Decramer et al, 2012).*** Understanding the genetic predisposition of COPD is essential to develop personalized treatment regimens (***Shapiro, 2011***).

The beta$_2$-adrenergic receptor (β_2-AR) mediates various physiologic responses of the airways, including bronchodilation, anti-inflammatory actions, mucociliary clearance, and vascular endothelial permeability (***Yang et al., 2007; Augusto et al., 2010; Marson et al., 2012***). Alterations in the *ADRB2* gene have a significant physiologic role in regulating responses to exogenous β_2-agonists (***Al-Rubaish, 2011; Karam et al., 2013***).

This case-control study was conducted to assess the genetic association between *ADRB2* gene polymorphisms (Arg16Gly and Gln27Glu) and COPD in Egyptian patients, and to analyze their impact on the clinical outcome and therapeutic response.

The study included 61 COPD patients and 54 healthy controls. Patients were recruited from Abasseyiah Chest Hospital, Cairo, and Port-Said Chest Hospital, Port-Said, Egypt. They were diagnosed based on history, physical examination, and spirometric data, and FEV_1/FVC ratio <70%, according to the Global Initiative for Chronic Obstructive Lung Disease criteria (***Mervyn, 2009; GOLD, 2013***).

Severity of dyspnea was assessed based on Modified Medical Research Council (MMRC) scale. Patients' symptoms and the impact of COPD disease on health status was represented by COPD Assessment Test (CAT) score. History of medication use in COPD patients (oral and inhaled corticosteroids, and bronchodilators) was obtained. Brinkman smoking index and BMI were calculated in all participants. Chest radiographs were evaluated in all the subjects to exclude other causes of airflow limitation. Of the study participants, spirometry was performed to 43 patients and 35 controls before and after salbutamol inhalation.

DNA was extracted from whole blood using QIAamp DNA Blood Mini kit. Genotyping of the polymorphisms at positions 16 (Arg16Gly; *rs1042713*) and 27 (Gln27Glu; *rs1042714*) was performed by Real-Time PCR technology, using TaqMan® genotyping assays for both SNPs (*Applied Biosystem, assay ID C__2084764_20 and C__2084765_20*), to detect the presence of one or both alleles in each sample (***Augusto et al., 2010; Al-Rubaish, 2011***).

In the present study, we found that Gly_{16} and Gln_{27} represented 57% and 70% of the whole study population, respectively. Correspondingly, Arg_{16}/Gly_{16} and Gly_{16}/Gly_{16} at codon 16 and Gln_{27}/Gln_{27} and Gln_{27}/Glu_{27} at codon 27 were the most frequent genotypes. Haplotype analysis detected the presence of three haplotypes only in Egyptian population; Arg_{16}/Gln_{27}, Gly_{16}/Gln_{27}, and Gly_{16}/Glu_{27}.

ADRB2 polymorphisms among COPD patients and healthy controls, in the current study, demonstrated that Arg_{16}/Arg_{16} genotype was 4.1 times more likely than Arg_{16}/Gly_{16} and Gly_{16}/Gly_{16} to develop COPD. For Gln27Glu polymorphism, Gln_{27}/Gln_{27} was significantly the most predominant genotype in COPD patients; those carrying this genotype were 3.4 more likely to develop COPD than their counterparts. While in the healthy control group, the

heterozygote Gln_{27}/Glu_{27} genotype was significantly the most prevalent genotype.

Haplotype-based analysis also demonstrated that homozygotes for Arg_{16}/Gln_{27} haplotype were associated with more than 4 times increased risk of developing COPD, but if heterozygous with other haplotype combinations did not show any association with disease risk. Gly_{16}/Gln_{27} haplotype did not influence the susceptibility for developing COPD in our Egyptian population. However, the carriage rate of Gly_{16}/Glu_{27} haplotype was protective; with lower frequency in COPD patients.

Concerning the association between *ADRB2* polymorphisms and disease severity among our COPD patients, individuals with Arg_{16} genotypes and haplotypes had more severe grades of dyspnea, more frequent exacerbations, and higher impact of COPD disease on health status. However, genetic analysis at position 27 among our patients revealed that the Glu_{27} allele was significantly associated with better CAT scores and low impact of COPD disease on health status.

Baseline pulmonary function of our COPD patients, in our study, showed no differences in FEV_1 among patients with various *ADRB2* genotypes at position 16 and 27. In addition, there was no effect of different haplotype combinations on FEV_1.

On examining bronchodilator response (BDR) after salbutamol inhalation among our COPD patients, we found no effect on Arg16Gly polymorphism. As for Gln27Glu polymorphism, our results showed an interaction between Gln27Glu genotypes and BDR to salbutamol, with patients with Gln_{27}/Gln_{27} exhibiting the smallest response, patients with Gln_{27}/Glu_{27} an intermediate response, and patients with Glu_{27}/Glu_{27} the best response. Similarly, patients with Glu_{27} allele and those without Gln_{27} allele had significantly better BDR to salbutamol compared to their counterparts.

Haplotype analysis, in the present study, also showed better response in patients carrying Gly_{16}/Glu_{27} and those not carrying Gly_{16}/Gln_{27} haplotypes, with no dose-response relationship according to their copy number in the individual.

In summary, our results demonstrated that both Arg16Gly and Gln27Glu polymorphisms were associated with COPD prevalence, severity, and therapeutic response to β_2-adrenergic agonists in Egyptian population. Screening COPD patients for these genetic determinants during patient's assessment would guide the best medication choice in each patient, which may ultimately enhance care and improve health outcomes.

CONCLUSIONS

Genetic analysis of the two *ADRB2* polymorphisms revealed that Gly_{16} and Gln_{27} were the most prominent alleles, representing 57% and 70% of the Egyptian population, respectively. Only three haplotypes were detected; Arg_{16}/Gln_{27}, Gly_{16}/Gln_{27}, and Gly_{16}/Glu_{27}.

For disease susceptibility, genotypes and haplotypes homozygous for Arg_{16} and Gln_{27} were more likely to develop COPD. However, individuals carrying Glu_{27} allele conferred protection against COPD development.

For disease severity, genotypes and haplotypes containing Arg_{16} allele were also associated with higher grades of dyspnea, more frequent exacerbations, and more COPD symptoms. However, Gln27Glu polymorphism showed no influence on disease outcome.

Bronchodilator response after salbutamol inhalation showed no association with Arg16Gly polymorphism. In contrast, COPD patients carrying Glu_{27} allele in Gln27Glu polymorphism and those not carrying Gln_{27} had better bronchial airway responsiveness to β_2-agonists.

These results suggested that the Arg16Gly and Gln27Glu polymorphisms of the *ADRB2* gene, individually and in combination, play an important role in COPD among Egyptian population.

RECOMMENDATIONS

- The inheritance of Arg_{16} and Gln_{27} alleles was associated with poor clinical outcome and therapeutic response in Egyptian COPD patients, thus those patients will need more ongoing monitoring and assessment, and they may require additional lines of treatment to control disease symptoms.
- The role of *ADRB2* polymorphisms in the development and progression of COPD should be further investigated by large population-based studies and perhaps in other geographical areas within Egypt.
- Whether the Arg16Gly and Gln27Glu polymorphisms contributes directly to the pathogenesis of COPD or acts as a marker for a linked gene needs to be investigated.
- Further prospective validation will be needed to advance the mechanistic understanding of β_2-adrenergic polymorphisms and their association with clinical features of COPD.
- Future researches are needed to determine the interaction between *ADRB2* genetic polymorphisms and phenotypic heterogeneity of COPD spectrum.

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APPENDIX I

GLOBAL INITIATIVE FOR CHRONIC OBSTRUCTIVE LUNG DISEASE (GOLD) CRITERIA FOR COPD

GOLD definitions

Gold defines COPD as "a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases" (***Lacoma et al., 2009***).

A clinical diagnosis of COPD should be considered in any patient who has dyspnea, chronic cough or sputum production, and/or a history of exposure to risk factors for the disease. The diagnosis should be confirmed by spirometry. GOLD definition for airflow limitation is an FEV_1/FVC ratio of less than 70%. Whereas, airflow reversibility, spontaneously, in response to an inhaled bronchodilators, or in response to oral or inhaled steroids, as an FEV_1 increase of 200 ml and 12% improvement above baseline FEV_1 (***Mannino, 2001; Mervyn, 2009***).

GOLD staging system for COPD severity

The GOLD criteria classify COPD into 4 stages based on the patient's level of symptoms, the severity of spirometric abnormalities, and the presence of complications, Table (26).

Table 26. GOLD staging system for COPD severity.

Stage	Lung function characteristics*	Characteristics
0: at risk	Normal spirometry	Chronic symptoms (cough, sputum)
I: mild COPD	$FEV_1/FVC < 70\%$ $FEV_1 \geq 80\%$ predicted	Usually chronic cough and sputum production
II: moderate COPD	$FEV_1/FVC < 70\%$ $50\% \leq FEV_1 < 80\%$ predicted	Progression of symptoms; shortness of breath on exertion
III: severe COPD	$FEV_1/FVC < 70\%$ $30\% \leq FEV_1 < 50\%$ predicted	Greater shortness of breath; repeated exacerbations which have an impact on patients' quality of life
IV: very severe COPD	$FEV_1/FVC < 70\%$ $FEV_1 < 30\%$ predicted OR $FEV_1 < 50\%$ predicted + chronic respiratory failure #	At this stage, quality of life is appreciably impaired and exacerbations may be life-threatening

Reference: adapted from Global Initiative for COPD. Global strategy for diagnosis, management, and prevention of COPD. 2007. Available from http://www.goldcopd.org.

* All lung function measurements are post-bronchodilator values.

Respiratory failure: arterial partial pressure of oxygen (PaO2) < 8.0 kPa (60 mmHg) with or without arterial partial pressure of Carbon dioxide (PaCO2) > 6.7 kPa (50 mmHg) while breathing air at sea level.

FEV_1: forced expiratory volume in one second; FVC: forced vital capacity.

Exacerbations

An acute exacerbation of the disease is defined as the presence of at least 2 major, or 1 major and 1 minor, symptom, on 2 or more consecutive days. Worsening dyspnea, increase in sputum volume, and increased sputum purulence are considered major symptoms, and increased cough or chest tightness, wheeze, sore throat, and coryza are considered minor symptoms (***Mokry et al., 2008***).

APPENDIX II

STRUCTURED INTERVIEW FOR COPD PATIENTS AND CONTROLS

CODE NO (……)

Personal history

1. Birthday
   - Phone no:
2. Gender ○ Female ○ Male
   - Address:
3. Marital status ○ Single ○ Married ○ Widow ○ Divorced
4. Race ○ Semitic white ○ Black Nubian ○ Others

History of exposure to risk factors (sign if any present)

Smoking

5. Smoking status ○ Current-smoker ○ Former-smoker ○ Never-smoker ○ Secondhand smoker
6. Lifetime smoking ○ Years ---------- ○ Packs ----------
7. Type of smoking ○ Cigarette ○ Cigar ○ Pipe

Occupational dusts and chemicals

8. Occupational status ○ Unemployed ○ Employed ○ Retired/disabled
9. Usual occupation --------- No of years ---------
10. Dust exposure --------- Total years ---------
11. Fumes exposure --------- Total years ---------

Outdoor and indoor pollution

12. Use biomass fuel for cooking and heating (as wood, coal, straw, and crop residues)
13. Outdoor air pollution from motor vehicle emissions and other fossil fuel combustion
14. Chemical fumes and dust from the environment

Other risk factors

15. History of pulmonary TB
16. Chronic asthma
17. RTI during childhood
18. Poor socioeconomic status ○ Low income ○ Poor housing conditions ○ Low educational level

Present history (sign if any present)

Chest symptoms

19. Shortness of breath ○ at rest * ○ during exercise * ○ *MMRC* stage ( )
20. Coughing *
21. Sputum production * ○ Amount ○ Color
22. Wheezing and chest tightness
23. Fatigue

General symptoms

24. Exclude infection ○ Fever ○ Chills ○ Sweat ○ Purulent discharge
25. Exclude other CLD or lung cancer ○ Hemoptysis ○ Not respond to ttt
26. Exclude heart disease ○ Chest pain ○ Palpitation ○ Ankle swelling ○ PND
27. Exclude GERD ○ Indigestion ○ Heartburn ○ Water brush
28. Exclude systemic dis. or malignancy ○ Anemia ○ Anorexia ○ Recent weight loss

Impact on patient's life

29. Limitation of activity ○ Strenuous activities * (stairs, hurrying, sports) ○ Moderate activities * (walking, housework, carrying things) ○ Home daily activities * (dressing, washing) ○ Social activities * (talking, being with children, visiting friends)
30. Economic impact ○ Missed work
31. Mental status ○ Concern about getting cold or breathing ○ Depressed because of breathing

	getting worse *		problems *	
Complications				
32. History of exacerbations	○ Frequency ---------- /year			
33. Chest complications	○ Resp. infection	○ Pneumonia	○ Pneumothorax	○ Respiratory failure
	○ Lung cancer	○ Bronchiectasis	○ Atelectasis	
34. Cardiovascular disease	○ Pulmonary HT	○ RHF	○ Cor pulmonale	○ Arrhythmia
	○ CAD			
35. Psychiatric morbidity	○ Depression	○ Anxiety		
36. Other system affection	○ Sk. muscle wasting	○ Sleep-related dis.	○ Stroke	○ Malnutrition
	○ 2ry polycythemia			

Past history (sign if any present)

37. History of chest illness	○ Asthma	○ TB	○ Pneumonia	○ Respiratory infections in childhood	
	○ Attacks of bronchitis	○ Hay fever	○ Allergy	○ Sinusitis or nasal polyps	
38. Contact with TB patient					
39. Presence of comorbid disease	○ Cardiovascular dis	○ Hypertension	○ Diabetes	○ Osteoporosis	○ Renal disease
	○ Cerebrovascular dis	○ Malignancies	○ Depression	○ Arthritis	○ Liver disease
	○ Cachexia: ↓ FFM	○ Anemia	○ Hyperlipidemia	○ GERD	○ Bilharziasis
40. Hospitalization for respiratory disorder					
41. Chest operations					
42. Chest injuries					
43. Radiotherapy					

COPD management history (sign if any present)

44. Disease diagnosed since	---------- years			
45. No of respiratory medications	----------			
46. Medications used	○ None	○ SABA	○ Anticholinergic	○ ICS
	○ O_2 therapy	○ LABA	○ Methylthanthines	○ OCS
	○ Combined SABA and anticholinergic		○ Combined LABA and steroids	
47. Other pharmacological ttt	○ Vaccines	○ Mucolytics	○ Antioxidants	○ Vasodilators
	○ Antibiotics	○ Antitussives	○ Immunorgulators	○ Narcotics
	○ α_1-AT augmentation therapy			
48. Lifetime OCS use	------ years			
49. Ventilator support	○ Yes	○ No		
50. Pulmonary rehabilitation	○ Exercise training	○ Nutritional counseling	○ Education	
51. Surgery	○ Bullectomy	○ Lung volume reduction surgery	○ Lung transplantation	
52. Drugs for other purpose				

Family history (sign if any present)

53. Consanguinity	○ Yes	○ No	
54. Heridofamilial diseases	○ Yes	○ No	Identify ----------
55. History of COPD	○ Yes	○ No	
56. History of chronic lung disease	○ Yes	○ No	Identify ----------
57. History TB, DM, or hypertension	○ Yes	○ No	

General Appearance

58. Appearance or look	○ Toxic	○ Non-toxic	
59. Body built	○ BMI = -------kg/m^2	○ Weight = ----- Kg	○ Height = ----- cm
60. Decubitus			
61. Mental state	○ Conscious level	○ Mood	○ Intelligence

Vital Signs

62. Pulse (rate, rhythm, volume, tension, equality, peripheral pulsation, condition of wall, special characters)
63. Respiratory rate and depth
64. Temperature
65. Blood pressure (Pulsus paradoxus)

General physical examination

66. Eye examination	○ Puffiness ○ Subconjunctival he	○ Jaundice ○ Ptosis
67. Nose examination	○ Polyps for allergic rhinitis ○ Overworking ala nasi	○ Blood stained nasal discharge ○ Granuloma
68. Mouth examination	○ Pursing of lips during expiration	
69. Neck examination	○ Shape and position ○ Overacting sternomastoid muscles ○ Cervical LN	○ Neck veins ○ Carotid pulsations ○ Thyroid swelling
70. Skin examination	○ Pallor ○ Cyanosis	○ Jaundice ○ Pigmentation
71. Upper extremity examination	○ Clubbing ○ Edema ○ Axillary LNs	○ Flapping tremors ○ Fine tremors
72. Abdominal examination	○ Displaced liver ○ Ascites ○ Epigastric pulsation	○ Organomegaly ○ Dilated veins ○ Tenderness
73. Lower extremity examination	○ Clubbing ○ Edema	○ Signs of DVT (tenderness, hotness) ○ Inguinal and popliteal LNs
74. Heart examination	----------	

Local chest examination

Combined inspection and palpation

75. Skin	○ Scars / sinus / ulcer	○ Pigmentation	○ Dilated veins
76. Shape			
77. Movement			
78. Accessory muscles of respiration			
79. Pulsations			
80. Trachea			
81. Tenderness			
82. TVF			
83. Palpable adventitious sounds			

Percussion

84. Liver
85. Right border of heart
86. Base of heart at 2nd ICS
87. Bare area
88. Traub's area
89. Kronig's isthmus
90. Lung proper

Auscultation

91. Heart sounds and murmurs			
92. Breath sounds			
93. Adventitious sounds	○ Rhonchi	○ Crackles	○ Pleural rub
94. Vocal resonance	○ Bronchophony	○ Aegophony	○ Whispering pectoriloquy
95. Special auscultatory signs			

Investigation (sign if any present)

Laboratory findings

- CBC
- ABG ○ PaO_2 ○ $PaCO_2$ ○ SaO_2 ○ pH
- Renal profile
- Hepatic profile
- Electrolytes
- Others

Microbiology

Sputum culture and sensitivity

Radiology

- Chest X-ray
- High resolution CT
- ECG
- Echocardiography
- Others

Spirometry

Pre-bronchodilator	**Post-bronchodilator**	**Bronchodilator response**
▪ FEV_1	▪ FEV_1	▪ BDRABS
▪ FVC	▪ FVC	▪ BDRBASE
▪ FEV_1/ FVC	▪ FEV_1/ FVC	▪ BDRPRED
▪ $FEV_{1\%}$	▪ $FEV_{1\%}$	
▪ PEF	▪ PEF	

Other tests

- $Alpha_1$-antitrypsin

APPENDIX III

MODIFIED MEDICAL RESEARCH COUNCIL (MMRC) DYSPNEA SCALE

The modified MMRC scale uses a simple grading system to assess a patient's level of dyspnea from none (Grade 1) to almost complete incapacity (Grade 5) (***Fletcher et al., 1959; Stenton, 2008***), Table (27).

Table (27). mMRC Dyspnea Scale.

Grade	Degree of breathlessness related to activities
0	Breathless only with strenuous exercise.
1	Short of breath when hurrying on level ground or walking up a slight hill.
2	Walks slower on level ground than people of the same age because of breathlessness, or has to stop for breath when walking at own pace.
3	Stops for breath after walking about 100 meters or after a few minutes on level ground.
4	Too breathless to leave the house, or breathless when dressing or undressing.

APPENDIX IV

COPD ASSESSMENT TEST ™ (CAT)

The COPD Assessment Test (CAT) is a questionnaire for COPD patients, developed in 2009. It is used to measure the impact of COPD on health-related quality of life and to aid patient-physician communication (***Van der Molen et al., 2013***). The CAT is a simple and easy-to-use questionnaire that distinguishes between patients of different degrees of COPD severity (***Jones et al., 2011***). It is an eight-item questionnaire suitable for routine clinical use that shows reliability and validity in stable and exacerbating COPD (***Jones et al., 2012***). The eight items in the CAT cover cough, phlegm, chest tightness, capacity for exercise and activities, confidence leaving home, sleep quality, and energy levels (***Van der Molen et al., 2013***), Figure (27). Each item is scored from 0 (best) to 5 (worst). The scaling range is from 0 to 40. Higher scores denote a more severe impact of COPD on a patient's life (***Dodd et al., 2011***), Table (28).

Table (28). Broad clinical picture of the impact of COPD by CAT score (*Jones et al., 2011*).

CAT score	Impact level	Clinical picture
>30	Very high	Their condition stops them doing everything they want to do and they never have any good days. If they can manage to take a bath or shower, it takes them a long time. They cannot go out of the house for shopping or recreation, or do their housework. Often, they cannot go far from their bed or chair. They feel as if they have become an invalid.
>20	High	COPD stops them doing most things that they want to do. They are breathless walking around the home and when getting washed or dressed. They may be breathless when they talk. Their cough makes them tired and their chest symptoms disturb their sleep on most nights. They feel that exercise is not safe for them and everything they do seems too much effort. They are afraid and panic and do not feel in control of their chest problem.
10-20	Medium	COPD is one of the most important problems that they have. They have a few good days a week, but cough up sputum on most days and have one or two exacerbations a year. They are breathless on most days and usually wake up with chest tightness or wheeze. They get breathless on bending over and can only walk up a flight of stairs slowly. They either do their housework slowly or have to stop for rests.
<10	Low	Most days are good, but COPD causes a few problems and stops people doing one or two things that they would like to do. They usually cough several days a week and get breathless when playing sports and games and when carrying heavy loads. They have to slow down or stop when walking up hills or if they hurry when walking on level ground. They get exhausted easily.
5		Upper limit of normal in healthy non-smokers.

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