Beta-2 Adrenergic Receptor Gene Polymorphisms in Chronic Obstructive Pulmonary Disease

A Thesis Submitted in Partial Fulfillment of the Master Degree in Chest diseases

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بسم الله الرحمن الرحيم

" وَأَنزَلَ اللَّهُ عَلَيْكَ
الْكِتَابَ وَالْحِكْمَةَ
وَعَلَّمَكَ مَا لَمْ تَكُنْ
تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ
عَلَيْكَ عَظِيمًا "

النساء ١١٣

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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
Arg	Arginine
Arg16Gly	Amino acid substitution from arginine to glycine at codon 16
Arg/Arg	Homozygote for Arg allele
Arg/Gly	Heterozygote with Arg allele and Gly allele
BDR	Bronchodilator response
BDRABS	Absolute change in FEV1
BDRBASE	Change in FEV1 as a percent of baseline FEV1
BDRPRED	Change in FEV1 as a percent of predicted FEV1
BHR	Bronchial hyper-responsiveness
BMI	Body mass index
BODE	Body-mass index, airflow obstruction, dyspnea, and exercise capacity
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

FEV_6	Forced expiratory volume after six seconds
FVC	Forced vital capacity
GDP	Guanosine diphosphate
GERK2	G protein–coupled receptor kinases
Gi	Inhibitory G protein
Gln	Glutamine
Gln27Glu	Amino acid substitution from glutamine to glutamic acid at codon 27
Gln/Gln	Homozygote for Gln allele
Gln/Glu	Heterozygote with Gln allele and Glu allele
Glu	Glutamic acid
Glu/Glu	Homozygote for Glu allele
Gly	Glycine
Gly/Gly	Homozygote for Gly allele
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
Nrf2	The nuclear factor erythroid 2-related factor
Nt	Nucleotide
OR	Odds ratio
$PaCO_2$	Partial pressure of carbon dioxide in arterial blood
PaO_2	Partial pressure of oxygen in arterial blood
PCR	Polymerase chain reaction
PDE	Phosphodiesterase
PEFR	Peak expiratory flow rate
PFT	Pulmonary function test
PKA	Protein kinase A
RV	Right ventricular
SABA	Short-acting $beta_2$-agonist
SaO_2	Oxygen saturation in arterial blood
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., 2006; 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., 2003; 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; 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.