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

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

By Mohammad Hosny Mohammad Hussein

Supervisors

Professor Khaled Eid Sobhy

Professor of Chest Diseases
Faculty of Medicine
Cairo University

Dr.
Irene Mohamed Sabry

Associate Professor of Chest Diseases
Faculty of Medicine
Cairo University

Dr.
Ahmed Taher El Serafi

Lecturer in Biochemistry
Faculty of Medicine
Suez Canal University

Faculty of Medicine Cairo University 2014

Candidate contact information

Candidate name Mohammad Hosny Mohammad Hussein

M.B.B.CH. 2000 Faculty of Medicine Cairo University

Address Portsaid El-Mohandsean building A/613

Tel 01001842212 *Fax* 0643219109

Email Hosny.mh@gmail.com

Date of registration for Master Degree: 2006

Investigators contact information

Principal investigator Professor Khaled Eid Sobhy

Department of Chest Diseases

Faculty of Medicine Cairo University

Address Chest disease department (unit 3) Kasr Alainy, Cairo

university Hospital

Tel 01006002597

Email

Other investigators Dr. Irene Mohamed Sabry

Associate Professor of Chest Diseases

Faculty of Medicine Cairo University

Address Chest disease department (unit 3) Kasr Alainy, Cairo

university Hospital

Tel 01224109776

Email Irene.sabry@yahoo.com

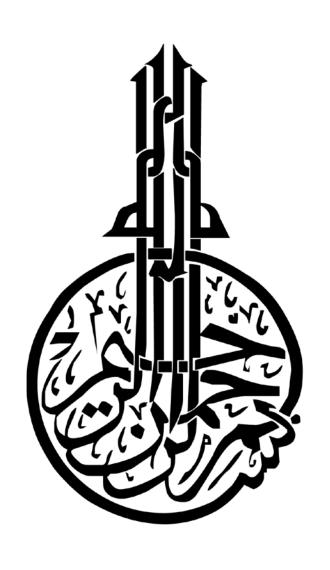
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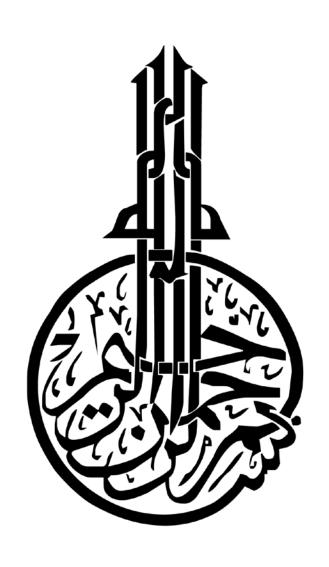
Lecturer in Biochemistry Faculty of Medicine Suez Canal University

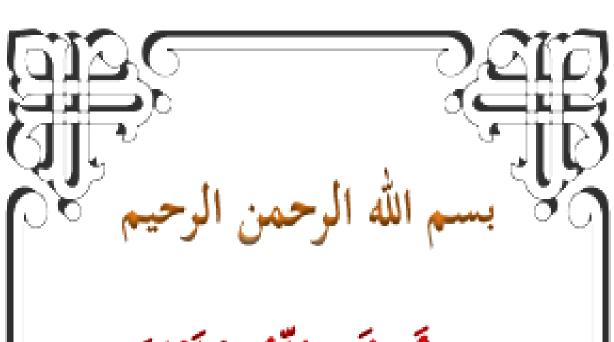
Address Biochemistry department, Suez Canal university Hospital

Tel 01223461901 *Fax* 066 3241750

Email <u>ahmserafi@yahoo.co.uk</u>







" وَأَنزَلَ اللّهُ عَلَيْكَ الْكِتَابَ وَالْحِكْمَةَ وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللّهِ عَلَيْكَ عَظِيمًا " عَلَيْكَ عَظِيمًا "



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List of Abbreviations

 χ^2 Chi-square test

AC Adenylyl cyclase

ADRB2 Beta₂-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

Ca²⁺ calcium ion

cAMP Cyclic adenosine monophosphate

CI Confidence interval
CO Carbon monoxide
CO₂ 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

FEV₁ Forced expiratory volume after one second

FEV₆ 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 beta₂-agonist

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

PaCO₂ Partial pressure of carbon dioxide in arterial blood

PaO₂ 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 beta₂-agonist

SaO₂ 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 Beta₂-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). Beta₂-adrenergic agonists (β_2 -agonists) are the most widely bronchodilators used to treat airflow limitations associated with COPD. Interindividual 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 beta₂-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): Glu₂₇ is almost always associated with Gly₁₆, whereas Gln₂₇ is associated with either Arg₁₆ or Gly₁₆ (*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 beta₂-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.