Association Between Gene Polymorphism of IL13/IL-Rα Signaling Pathway and Severity of Bronchial Asthma

THESIS

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List of Abbreviations

Ab	Antibody
ADAM	A Disintegrin and Metalloproteinase Domain
Arg	Arginine
BA	Bronchial Asthma
BAL	Bronchoalveolar Lavage
BHR	Bronchial hyperresponsiveness
Bsa	Bacillus stearothermophilus
CAT	Cambridge Antibody Technology
CBA	Chronic Bronchial Asthma
CD	Cluster of Differentiation
CI	Confidence Interval
CRH	Cytokine Receptor Homology
DEPC	Diethyl Pyrocarbonate
dNTPs	Deoxy Nucleoside Triphosphate
EB	Ethidium Bromide
ECP	Eosinophil Cationic Protein
EIA	Exercise Induced Asthma
EIB	Exercise Induced Bronchospasm
ELISA	Enzyme Linked Immunosorbent Assay
EPR	Expert Panel Report
EPX/EDN	Eosinophil protein X/Eosinophil Derived Neurotoxin
ER	Endoplasmic Reticulum
ERK	Extracellular signal–Regulated Kinases
ETS	Environmental Tobacco Smoke
FEV1	Forced Expiratory Volume in the first second
FVC	Forced Vital Capacity
GCs	Glucocorticoids
GERD	Gastro Esophageal Reflux Disease
GINA	Global Initiative of Asthma
Gln	Glutamine
Glu	Glutamate
Gly	Glycine
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
gp	Glycoprotein
HA	Hemagglutinin
HDLC	High Density Lipoprotein Cholesterol

His	Histidine
HRVC	Human rhinovirus C
IAV	Influenza A virus
ICSs	Inhaled corticosteroids
Ig	Immunoglobulin
IL	Interleukin
IL-4αR	Interleukin 4 alpha Receptor
INF	Interferon
iNOS	inducible Nitric Oxide Synthase
IPA	Invasive Pulmonary Aspergillosis
IRS	Insulin Receptor Sustrate
JAK	Janus Kinase
kb	Kilobase
KDa	Kilodalton
LABAs	Long-Acting Beta2 agonists
Leu	Leucine
LPS	Lipopolysaccharides
Lys	Lysine
Maf	Musculo aponeurotic fibrosarcoma
MCP	Monocyte Chemoattractant Protein
MHC	Major Histocompatability Complex
MMP	Matrix Metalloproteinase
N	Neuraminidase
NAEPP	National Asthma Education and Prevention Program
NKT	Natural Killer T cells
NSAID	Non-Steroidal Anti-Inflammatory Drug
OR	Odds Ratio
P	Pseudomonas
PAP	Pulmonary Alveolar Proteinases
PEFR	Peak Expiratory Flow Rate
PGD	Prostaglandin D
Phe	Phenylalanine
PI	Phosphoinositol
RANTES	Regulated on Activation, Normal T cell Expressed and Secreted
RFLP	Restriction Fragment Length Polymorphism
RNASE	Ribonuclease
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction

S	Staphyloccocus
S.D.	Standard Deviation
SABA	Short Acting β Agonists
SCF	Stem Cell factor
Ser	Serine
siRNAs	small interfering RNAs
SNP	Single Nucleotide Polymorphism
STAT	Signal Transducer and Activator of Transcription
TGF	Transforming Growth Factor
TH	T Helper Cell
T-h	T-helper cell
Thr	Threonine
TNF	Tumor Necrosis Factor
TSLP	Thymic Stromal Lymphopoietin
Tyr	Tyrosine
UBA	Uncontrolled Bronchial Asthma
WCF	Weak Culture Filtrate
WHO	World Health Organization

ABSTRACT

Different genes are associated with categorical classifications of asthma severity. However, continuous outcomes should be used to catch the heterogeneity of asthma phenotypes and to increase the power in association studies. The aim of our work is to investigate whether there is association between single nucleotide polymorphism (SNPs) in 3 candidate genes of IL-13/IL-4Rα signaling pathway and the severity of bronchial asthma. Sixty subjects were enrolled in this study divided into 3 main groups: healthy control group (20 subjects), patients with severe bronchial asthma (20 subjects) and patients with mild bronchial asthma (20 subjects). Each group is subdivided into smokers and non smokers. **IL-13 C1923T, IL-4 C–590T and IL-4 RA 175V** were assessed using Restriction Fragment Length Polymorphism (RFLP). Results revealed that polymorphism of IL-13 C1923T genotypes and T allele as well as polymorphism of IL-4 R 175V genotypes and G allele are correlated to severity of asthma on one hand and with smoking status of patients on the other hand. These data suggest that genetic variants in IL-13/IL-4Rα signaling pathway may be involved in the development of severe asthma. In addition, a new pattern of biological interaction that affects the severity of asthma is described between SNPs of both IL-13 C1923T and IL-4 RA 175V and smoking.

Key words: IL-13/IL-4 signaling pathway, bronchial asthma, polymorphism, smoking.

Introduction and Aim of the Work

Introduction

Asthma is one of the most common chronic inflammatory lung diseases worldwide. Its incidence and mortality are increasing especially among children and its impact on society is substantial. Recent developments in molecular biology and genetics suggest that asthma is hereditary disorder based on several factors, including genes (**Leung et al., 2014)**.

For the past 10 years, many studies were concerned in the gene loci predisposing to asthma and other atopic disorders. Ober and Hoffjan reviewed 118 genes associated with asthma or atopy, and 25 of these have been duplicated in 6 or more studies and thus are believed to be the susceptible genes most likely to be associated with asthma and atopy **(Ober and Hoffjan, 2011).**

IL-13 also stimulates airways hyper responsiveness. Therefore, considering the importance of TH2 cytokines, specifically IL-13, in the complex immune response mounted by susceptible patients with allergic asthma, this cytokine is certainly a relevant target for asthma therapy. However, many other airway cell types and pathways are involved in the allergen immune response, and these pathways might be key in the expression of the different asthma phenotypes **(Ramirez et al., 2010).**

IL-4 is a cytokine that induces differentiation of naive helper T cells to Th2 cells. It has many biological roles, including the stimulation of activated B-cell and T-cell proliferation and the differentiation of B cells into plasma cells. IL-4 induces B-cell class switching to IgE, and up-regulates Major Histocompatibility Complex (MHC) class II production, decreases the production of Th1 cells, macrophages, Interferon (IFN) gamma, and dendritic cell IL-12 **(Sokol et al., 2008).**

A complex receptor system mediates the signaling of IL-13 and IL-4. Four heterodimeric receptor complexes have been identified to bind IL-4, IL-13, or both. The IL-4Ra subunit is a component of both the type I and type II receptors. Type I receptors are composed of the IL-4Ra subunit complexed with the common g chain. These type I receptors bind to IL-4 on cells of hematopoietic stem cell origin **(Hershey, 2003).**

Current asthma treatment with anti-inflammatory therapy does not appear to prevent progression of the underlying disease severity and therefore additional treatment options are needed.

We hypothesized that Single Nucleotide Polymorphisms (SNPs) of IL-**13 C1923T, IL-4 C–590T and IL-4 RA 175V** as well as smoking has an association with severity of bronchial asthma. In order to determine these associations we have conducted a cross-sectional study of healthy control group, patients with severe bronchial asthma with frequent exacerbations and patients with mild bronchial asthma.

Aim of the work

The aim of this work is to investigate whether there is association between SNPs in IL-13/IL-4 signaling pathway and the severity of bronchial asthma and to investigate whether smoking has an additive effect with these SNPs on asthma severity.