POLYMORPHISM OF THE CC16 GENE AND ITS ASSOCIATION WITH INCREASED RISK OF ASTHMA

Thesis

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ABSTRACT

Acute asthma is the most common diagnosis in children admitted to hospitals. Among the many genetic variations associated with asthma and asthma phenotypes linked to chromosome 11q13, CC16 is a candidate gene for involvement in the inherited predisposition to asthma. CC16 is the most abundant protein secreted into the airway and it diffuses passively into the serum where it mirrors changes occurring in the lung. It functions as an anti-inflammatory and immunomodulatory agent where it inhibits neutrophil and monocyte chemotaxis, phospholipaseA_2 and IFNγ. It has been proposed as a marker for respiratory epithelial injury, non small cell carcinoma and renal tubular dysfunction. CC16 gene has a functional promoter region polymorphism (A38G). The CC16 38A allele is associated with decreased CC16 plasma levels and increased incidence of asthma.

This study is an attempt to assess the genetic association of the variants of the CC16 gene in relation to asthma in an Egyptian pediatric population. Forty four asthmatic children (26 males and 18 females), aged 4.5-14 years (mean age 7.73±2.86 years), with mild to severe persistent asthma were chosen. Also 21 age and sex matched children were enrolled as controls. The blood eosinophilic counts, total serum IgE levels, PEFR and were determined for all subjects. Genotyping of exon1 of CC16 gene was done using RFLP-PCR technique. CC16 A38G SNP did not show significant difference in pediatric asthmatic patients compared to the control group. Furthermore, no differences were found in these genotypes as regards atopy, atopy-related phenotypes, IgE level and blood eosinophilic count. However, significantly lower PEFR was found in the AA genotype compared to AG and GG genotypes. These findings may suggest that CC16 A38G polymorphism does not have disease susceptibility potential, but may be related to the severity of the disease.

Keywords:
Asthma, Atopy, Atopic asthma, Bronchial hyperresponsiveness, Clara cell, Clara cell protein 16, CC16, CC16 A38G polymorphism, CC16 genotypes, IgE, Nonatopic asthma, PEFR

LIST OF ABBREVIATIONS

AC	: Allergic conjunctivitis
AD	: Allergic dermatitis
AHR	: Airway hyperresponsiveness
APC	: Antigen presenting cells
AR	: Allergic rhinitis
ARDS	: Adult respiratory distress syndrome
ATS	: American Thoracic Society
BALF	: Bronchoalveolar lavage fluid
β_2AR	: βeta$_2$ adrenergic receptor/adrenoceptor
BHR	: Bronchial hyperresponsiveness
BOS	: Bronchiolitis obliterative syndrome
BPD	: Bronchopulmonary dysplasia
CAMP	: Cyclic adenosine monophosphate
CBC	: Complete blood count
CC16 SCGB	: CC16 secretoglobins
CC16	: Clara cell protein 16
CCR	: Receptor for CC chemokine
CCSP	: Clara cell secretory protein
CD	: Cluster of differentiation
COPD	: Chronic obstructive pulmonary disease
COX	: Cyclo-oxygenase
CX3CR	: Receptor for CX3C chemokine
CXCR	: Receptor for CXC chemokine
Da	: Daltons
DALY	: Disability Adjusted life-year
DNA	: Deoxyribonucleic acid
EBC	: Exhaled breath condensate
EBM	: Exaggerated bronchovascular markings
ECHRS	: European Community Respiratory Health Survey
ECP	: Eosinophil cationic protein
EDN	: Eosinophil derived neurotoxin
EDTA	: Ethylenediamine tetra-acetic acid
EIB	: Exercise-induced bronchospasm
EPO	: Eosinophil peroxidase
EPX	: Eosinophil protein X
FcεRI	: High affinity Fc receptors for IgE
FcεRII	: Low affinity Fc receptors for IgE
FeNO	: Fractional exhaled nitric oxide
FEV_1	: Forced expiratory volume in one second
FGF3	: Fibroblast growth factor 3
FVC	: Forced vital capacity

GATA3 : GATA-binding protein 3
GINA : Global Initiative for Asthma
GM-CSF : Granulocyte macrophage colony stimulating factor
GRO : Growth-related oncogene
GST : Glutathione S-transferase
HI : Hyperinflated
HLA : Human leukocyte antigen
HNF : Hepatocyte nuclear factor
Htm4 : Human haematopoietic cell-specific four-transmembrane protein
ICAM-1 : Intracellular adhesion molecule 1
ICS : Inhaled corticosteroids
IgAN : IgA nephropathy
IgE : Immunoglobulin E
IL : Interleukin
INFγ : Interferon gamma
ISAAC : International Study of Asthma and Allergies in Childhood
LABA : Long-acting $beta_2$-agonist
LTC4 : Cysteinyl-leukotrienes
LTRA : Leukotriene receptor antagonist
MAAS : Multicenter asthma study
MAPK : Mitosis-associated protein kinase
MBP : Major basic protein
MCP : Monocyte chemotactic protein
MDC : Macrophage-derived chemokine
MHC : Major histocompatibility complex
MIP : Macrophage inflammatory protein 1α
MUC2 : Mucin 2
NFAT : Nuclear factor of activated T cells
NF-κβ : Nuclear factor- κβ
NHLBI : National heart lung blood Institute
NK : Natural killer cells
PARC : Pulmonary and activation-regulated chemokine
PBT : Peribronchial thickening
PCR : Polymerase chain reaction
PDGF : Platelet derived growth factor
PEFR : Peak expiratory flow rate
PG : Prostaglandin
PLA_2 : Phospholipase A_2
RANTES : Regulated upon activation normal T cell expressed and secreted
RFLP : Restriction Fragment Length Polymorphism
RNA : Ribonucleic acid

RSV	: Respiratory syncytial virus
SABA	: Short-acting $beta_2$-agonist
SCG	: Secretoglobin
SD	: Standard deviation
SDS-PAGE	: Sodium-dodecyl-sulphate poly-acrylamide gel electrophoresis
SLE	: Systemic lupus erythematosus
SNP	: Single nucleotide polymorphism
SPT	: Skin prick test
TARC	: Thymus and activation-regulated chemokine
TBE	: Tris Borate EDTA (buffer)
TCR	: T-cell receptor
Th	: T-helper
TNFα	: Tumor necrosis factor alpha
U-EPX	: Urinary eosinophil protein X
UV	: Ultraviolet
VCAM-1	: Vascular cell adhesion molecule 1
VLA-4	: Very late antigen 4
XCR	: Receptor for XC chemokine,
YLD	: Years lost to disability
YLL	: Years of life lost to premature mortality

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INTRODUCTION

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Asthma is a chronic inflammatory disease of the airways, characterized by airway hyperreactivity, mucous hypersecretion, and airflow obstruction. It is the third leading cause of hospitalization among persons under 18 years of age in the United States, exceeded only by pneumonia and injuries **(Health Statistics Center, 2006)**. Despite recent advances, the genetic regulation of asthma pathogenesis is still largely unknown. Gene expression profiling techniques are well suited to study complex diseases and hold substantial promise for identifying novel genes and pathways in asthma; however, relatively few studies have been completed in human asthma **(Hansel and Diette, 2007)**.

Asthma is unlikely to be a single disease but rather a series of complex, overlapping individual diseases or phenotypes, each defined by its unique interaction between genetic and environmental factors. These conditions include syndromes characterized by allergens-exacerbated, nonallergic, and aspirin- exacerbated factors along with syndromes best distinguished by their pathologic findings (eosinophilic, neutrophilic, pauci-granulocytic), response to therapy (corticosteroid resistant), and natural history (remodeling prone). Additional phenotypes will almost certainly be identified as advances in genetics and other profiling methods are made and will be

accompanied by availability of clear biomarkers for distinguishing among them **(Borish and Culp, 2008)**.

Clara cell protein (CC16) is a 15.8-kDa protein secreted all along the tracheobronchial tree and especially in the terminal bronchioles where Clara cells are localized. Even though the exact in vivo function of CC16 remains to be clarified, evidence is accumulating that CC16 plays an important protective role in the respiratory tract against oxidative stress and inflammatory response. CC16, however, presents also a major interest as a peripheral lung marker for assessing the cellular integrity or the permeability of the lung epithelium. The serum concentration of the CC16 are decreased in subjects with chronic lung damage caused by tobacco smoke and other air pollutants as a consequence of the destruction of Clara cells. Asthmatic patients have lower circulating levels than those without asthma **(Shijubo et al., 1999a)**. By contrast, serum CC16 increases in acute or chronic lung disorders characterized by increased airways permeability. Although the clinical significance of these early epithelial changes detected by serum CC16 remains to be determined, these results clearly show that the assay in serum of lung secretory proteins such as CC16 represents a new noninvasive approach to evaluate the integrity of the respiratory tract **(Broeckaert et al., 2000)**.

The gene for CC16, located on chromosome 11q13, is a candidate for involvement in an inherited predisposition to asthma

because of its chromosomal location, the role of the CC16 protein in controlling airway inflammation, and differences in levels of the protein between asthmatics and healthy controls. A single-nucleotide polymorphism (SNP) in the CC16 gene (A38G) is described **(Laing et al., 1998a; Ohchi et al., 2004)**. Functional genetic variants of CC16 that influence protein expression might therefore contribute to asthma and asthma severity. **Laing et al. (1998a)**, described an association of the 38AA genotype with asthma and lower CC16 serum levels, while the 38G allele was associated with a decreased risk of developing childhood asthma.

AIM OF THE WORK