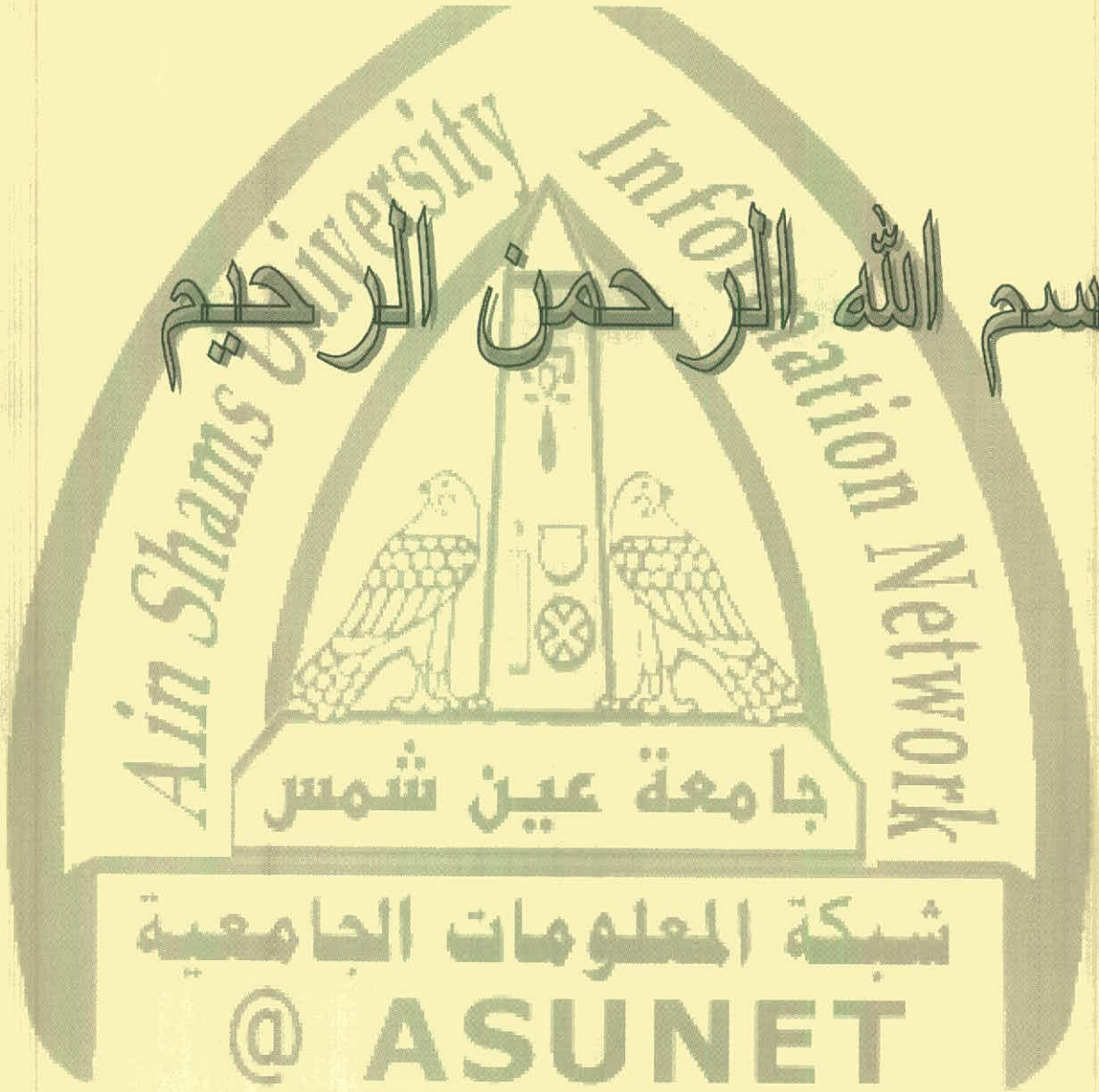
شبكة المعلومات الجامعية



بسم الله الرحمن الرحيم

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأفلام قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأفلام بعيدا عن الغبار

في درجة حرارة من ١٥-٢٥ مئوية ورطوبة نسبية من ٢٠-٤٠%

To be Kept away from Dust in Dry Cool place of 15-25- c and relative humidity 20-40%

بعض الوثائـــــــق الاصليـــــة تالفـــة

بالرسالة صفحات لم ترد بالاصل

616,24

Studies in the genetics of chronic obstructive pulmonary disease

(慢性閉塞性肺疾患の遺伝的要因の研究)

A dissertation submitted to the University of Tsukuba for partial fulfillment of the degree of

Doctor of Philosophy

in Pulmonary Medicine

By

Dr. Ahmed Elsayed Moustafa Hegab

Division of Pulmonary Medicine,

Doctoral Program in Medical Sciences for Control of Pathological Processes,

Graduate School of Comprehensive Human Sciences,

Japan

2005

TO WHOM IT MAY CONCERN

We herby certify that this is a typical copy of

the original Doctor Thesis of

Dr. Ahmed Elsayed Moustafa Hegab

Official Seal of Graduate School of
Comprehensive human sciences,
Medical Sciences Major, University
of Tsukuba

Prof. Tatsutoshi Goto

Dean of Graduate School of
Comprehensive human sciences,
Medical Sciences Major,
University of Tsukuba

يعتمد
المستشار الثقافي باليابان
أ.د/ كرم خليـــل سالم

MAR. 27. 2006

Contents

Abbreviations, 4
General introduction, 5
Aim of the present work, 11

Chapter 1
CLCA1 gene polymorphisms in chronic obstructive pulmonary disease

1.1. Introduction, 13
1.2. Materials and methods
    1.2.1. **Subjects, 14**
    1.2.2. **Screening the CLCA1 gene for polymorphisms, 15**
    1.2.3. **Genotyping, 15**
    1.2.4. **Statistical Analysis, 17**
1.3. Results, 17
1.4. Discussion, 20
1.5. Tables, 26
1.6. Figure legend, 36
1.7. Figure, 37

Chapter 2
Polymorphisms of IL4, IL13, and ADRB2 genes in COPD

2.1. Introduction, 39
2.2. Materials and methods
    2.2.1. **Subjects, 40**
    2.2.2. **Genotyping, 41**
    2.2.3 **Statistical analysis, 42**
2.3. Results, 43
2.4. Discussion, 45
2.5. Tables, 49

Chapter 3
Association analysis of tissue inhibitor of metalloproteinase2 gene polymorphisms with COPD in Egyptians

3.1. Introduction, 58
3.2. Materials and methods
3.2.1. Subjects, 58
3.2.2. Genotyping of TIMP2 polymorphisms, 58
3.2.3. Statistical analysis, 59
3.3. Results, 59
3.4. Discussion, 60
3.5. Tables, 62

Chapter 4
Promoter activity of human tissue inhibitor of metalloproteinase 2 gene with novel single nucleotide polymorphisms

4.1. Introduction, 65
4.2. Materials and methods
4.2.1. Subjects, 65
4.2.2. Isolation and DNA sequencing of *TIMP2* promoter region, 65
4.2.3. Genotyping, 66
4.2.4. Construction of *TIMP2* promoter/reporter gene plasmid, 66
4.2.5. Transient transfection and reporter gene assays, 67
4.2.6. Statistical analysis, 67
4.3. Results, 68
4.4. Discussion, 69
4.5. Tables, 72
4.6. Figure legend, 73
4.7. Figure, 74

Chapter 5
Polymorphisms of TNFα, IL1β, and IL1RN genes in chronic obstructive pulmonary disease

5.1. Introduction, 76
5.2. Materials and methods
5.2.1. Subjects, 77
5.2.2. Genotyping, 77
5.2.3. Statistical analysis, 78
5.3. Results, 79

5.4. Discussion, 80
5.5. Tables, 86

Summary and conclusion, 92
Acknowledgements, 94
Presentations, 96
Publication list, 97
References, 100
Published manuscripts, 116

Abbreviations

AAT: alpha-1-antitrypsin

ADRB2: β_2 adrenoceptor

AHR: airway hyperresponsiveness

bp: base pairs

CIs: confidence intervals

CLCA: chloride channel, calcium activated

cM: centi-Morgan

COPD: chronic obstructive pulmonary disease

FAM: 6-carboxyfluorescein

FEF: forced expiratory flow

FEV1: forced expiratory volume in one second

FVC: forced vital capacity

IL: interleukin

IL1RN: interleukin 1receptor antagonist

LD: linkage disequilibrium

MMPs: matrix metalloproteinases

Ors: odds ratios

RFLP: restriction fragment length polymorphism

SAGE: serial analysis of gene expression

SEAP: secreted form of human placental alkaline phosphatase

SNPs: single nucleotide polymorphisms

SSCP: single-stranded conformational polymorphism

TIMP2: tissue inhibitor of metalloproteinase2

TNF: tumor necrosis factor

VNTR: variable number of tandem repeat

General introduction

Chronic obstructive pulmonary disease (COPD) is characterized by a slowly progressive airflow limitation that is caused by peripheral airway inflammation and loss of lung elastic recoil resulting from parenchymal destruction. COPD has been recognized as a complex disease. Although cigarette smoking is the main risk factor for it, not all smokers develop COPD, suggesting that other factors contribute to the development of COPD. It is believed that interactions of genetic factors and environmental effects are involved in deciding who develops COPD. Cigarette smoking, air pollution, respiratory infections during childhood, latent adenoviral infections, and occupational exposure to vapors, gas, dust or fumes have been described as environmental risk factors [1-2]. Regarding the genetic risk factors, apart from alpha-1-antitrypsin (AAT) deficiency, the only well identified genetic risk factor for COPD; the genetic component in COPD is not a classical Mendelian pattern of inheritance but a complex pattern in which multiple genes are involved. Each of the genes has only a small contribution to the susceptibility [3]. The current challenge concerning complex human diseases is to elucidate their genetic mechanisms and to identify the susceptibility genes.

Several genetic methods are used for detecting genes responsible for the development of COPD; non-parametric linkage analysis, case-control association analysis and comprehensive gene expression profiling. These methods should be integrated with each other to accurately identify the polymorphisms of the candidate genes that are truly associated with COPD.

Evidence for a genetic basis to COPD

The idea that genetic factors play an important role in COPD was raised with the identification of familial aggregation [4]. The familial clustering was not due to the similar environment or smoking habits of the family members [5]. More recently, the risk to relatives of early-onset COPD probands for airflow obstruction and chronic bronchitis was studied. First degree relatives of early-onset COPD probands had significantly lower forced expiratory volume in one second (FEV1) and FEV1 / forced vital capacity (FVC) values than control subjects in spite of a similar smoking history [6]. Furthermore, it was demonstrated that non-smoking first degree relatives of early onset COPD probands have lower values of forced expiratory flow $(FEF)_{25-75}$ and FEF_{25-75}/FVC than controls [7], suggesting that genetic susceptibility to obstructive lung disease is independent of smoking. This susceptibility is magnified by exposure to cigarette smoke as suggested by the further decrements in FEF_{25-75} and FEF_{25-75}/FVC seen in the first degree relatives who smoke. Studies of pulmonary function in twins added even more evidences. It was shown that similarities in pulmonary function relate directly to genetic similarities and are consistent with a multi-factorial mode of inheritance [8-10]. In spite of these substantial evidences, we still do not know either the exact etiology or the relative roles of genes and the environment in COPD. However, systematic epidemiological, genetic and molecular studies are providing important insights into the development of COPD.

Strategies for identifying COPD susceptibility genes

There are three complementary types of genetic methods to identify genes

responsible for the development of adult-onset complex diseases; linkage analysis, association analysis and gene expression profiling with each approach having its advantages and limitations.

A) Linkage analysis

There are two approaches to locate predisposing genes within the concept of linkage analysis; parametric and nonparametric calculations. Parametric analysis is based on an explicit model of the inheritance of phenotypes and genotypes observed in pedigrees. It is the method of choice for the genetic mapping of diseases with Mendelian inheritance. By contrast, nonparametric analysis investigates whether affected relatives inherit a region identical-by-descent more often than expected by random Mendelian segregation. The simplest nonparametric analysis is the affected sib-pair analysis for a one-trait locus. The increasing availability of genetic markers (particularly microsatellites that contain nucleotide repeat sequences) that are closely interspersed throughout the genome makes it possible by nonparametric analysis to search the whole genome for regions that are associated with the affected individuals [11]. Since single nucleotide polymorphisms (SNPs) are distributed much more densely over the whole genome than microsatellites, it is expected that in the recent future, SNPs rather than microsatellites will be used in genome-wide linkage analysis for fine mapping of chromosomal regions associated with complex diseases. This nonparametric analysis is comprehensive and can locate chromosomal regions where any gene exerts a major effect on disease susceptibility. However, it has relatively low power and will fail to detect genes exerting only a moderate effect on risk of disease [12]. For example, if a disease susceptibility allele exerts a twofold

risk of the disease compared with the wild-type allele, 2500 to 300,000 families (depending on allele frequencies) would need to be typed to detect linkage to this gene, numbers which are not practically achievable. By genome-wide linkage analysis, several regions on chromosomes have been shown to have significant linkage to obstructive pattern of pulmonary function [13-19].

B) Association analysis

Association analysis involves selecting genes that are likely to be associated with the pathogenesis of COPD based on our understanding of its pathophysiology. Then polymorphisms in these candidate genes are investigated in a large number of unrelated patients and healthy ethnically matched controls. Significant differences in genotype or allele frequencies between the two groups suggest either that (a) the polymorphism predisposes one to the disease, (b) the polymorphism is in in linkage disequilibrium (LD) with a disease susceptibility gene, or (c) there is a confounding factor such as poor ethnic matching between the cases and controls. Association studies have greater power than linkage analysis. They can detect genes with a relative risk of 1.5 at nearly 80% probability if several hundred samples are collected [20]. However, since association studies examine much smaller regions than linkage analyses, many more markers would need to be typed to conduct a genome-wide association study. This is not possible with current technology. At present, association studies are limited to the investigation of candidate genes and regions identified in linkage analysis. As association studies are not comprehensive, the possibility that the most important genes have been overlooked cannot be excluded. Choosing candidate genes from areas spotted by linkage analysis might be the most fruitful practice