THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

Genomic and Genetic Analysis of Chronic Obstructive Pulmonary Disease and Inflammatory Bowel Diseases

by
Szilárd Póliska

Supervisor: Prof. Dr. László Nagy

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF MOLECULAR CELL AND IMMUNE BIOLOGY

Debrecen, 2011

TABLE OF CONTENTS

1 ABBREVIATIONS 4

2 INTRODUCTION 8

2.1 COPD 13
2.1.1 SYSTEMIC CO-MORBIDITIES OF COPD 15
2.1.1 INFLAMMATORY CELLS IN COPD 16
2.1.1.1 Neutrophils 17
2.1.1.2 Lymphocytes 18
2.1.1.3 Dendritic Cells 18
2.1.1.4 Epithelial cells 19
2.1.1.5 Monocytes/Macrophages 19
2.1.2 GENETIC RISK FACTORS OF COPD 21
2.1.2.1 Proteases-antiproteases 22
2.1.2.2 Antioxidants and xenobiotic metabolizing enzymes 23
2.1.2.3 Inflammatory mediators 24
2.1.2.4 Mediators of physiological processes in the respiratory tract 25
2.1.3 GENE EXPRESSION STUDIES IN COPD 26
2.2 IBD 27
2.2.1 PHENOTYPES OF IBD 29
2.2.2 GENETIC FACTORS OF IBD 29
2.2.2.1 Role of PPARγ in IBD 30
2.3 TRANSCRIPTIONAL REGULATION OF INFLAMMATION 31
2.3.1 NF-κB 31
2.3.2 AP-1 33
2.3.3 NUCLEAR RECEPTORS IN THE REGULATION OF INFLAMMATORY RESPONSE 33
2.3.3.1 GR 34
2.3.3.2 PPARs 35
2.3.3.2.1 PPARγ 35

3 AIMS 37

4 METHODS 38

4.1 STUDY POPULATION 38
4.1.1 INFORMED CONSENT 38
4.1.1.1 Inclusion/exclusion criteria for COPD study 38
4.1.1.2 Patient cohorts of IBD study 39
4.2 BAL PROCEDURE 40
4.3 ALVEOLAR MACROPHAGE SEPARATION 41
4.4 PERIPHERAL BLOOD MONOCYTE SEPARATION 41
4.5 RNA PROCESSING 41
4.6 MICROARRAY DATA ANALYSIS 42
4.7 TAQMAN REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION (RT-QPCR). 43
4.8 CANONICAL VARIATES ANALYSIS (CVA) 43

4.9 DNA ISOLATION **44**
4.10 SNP GENOTYPING **45**
4.11 IMMUNOHISTOCHEMISTRY **45**
4.12 STATISTICAL ANALYSIS OF SNP STUDY **45**

5 RESULTS **48**

5.1 GENE EXPRESSION ANALYSIS OF COPD **48**
5.1.1 EXPLORATORY MICROARRAY ANALYSES OF AMS OF BALF SAMPLES 48
5.1.1.1 Functional classification of COPD-specific genes 50
5.1.2 CONFIRMATION OF CANDIDATE GENES BY RT-QPCR 51
5.1.3 IDENTIFYING COPD-SPECIFIC GENE EXPRESSION SIGNATURES IN MOS 54
5.1.4 CORRELATION BETWEEN LUNG FUNCTION AND GENE EXPRESSION DATA 57
5.2 SNP ANALYSES IN CHRONIC INFLAMMATORY DISEASES **59**
5.2.1 COPD SNP STUDY 59
5.2.1.1 Analysis of SNPs of SERPINE2, TNF-α and SFTPB genes 60
5.2.1.2 Analysis of individual SNPs and haplotypes variants of EPHX1 gene 61
5.2.1.3 Analysis of PPARγ in COPD 62
5.2.2 IBD SNP STUDY 67
5.2.2.1 Disease association with individual SNPs: single locus analysis 67
5.2.2.2 Disease association with SNP haplotypes 69

6 DISCUSSION **73**

6.1 GENE EXPRESSION ANALYSIS OF COPD **74**
6.2 ANALYSES OF SNPS IN CHRONIC INFLAMMATORY DISEASES **76**
6.2.1 PPARγ SUSCEPTIBILITY IN CHRONIC INFLAMMATORY DISEASES 77
6.2.2 COPD STUDY 78
6.2.3 IDB STUDY 81

7 CONCLUSIONS **83**

8 ACKNOWLEDGEMENTS **85**

9 REFERENCES **86**

10 PUBLICATIONS **105**

10.1 PUBLICATIONS RELATED TO THE THESIS **105**
10.2 OTHER PUBLICATIONS **105**
10.3 FIRST AUTHOR POSTERS ON INTERNATIONAL MEETINGS **107**
10.4 PRESENTATIONS **107**

11 APPENDIX **108**

1 ABBREVIATIONS

15d-PGJ_2	15-deoxy-$D^{12, 14}$-prostaglandin J_2
5-ASA	5-aminosalicylic acid
α_1-AT	alpha$_1$-antitrypsin
β2AR	β_2-adrenergic receptor
ACTNB	beta-actin
ADRB2	β_2-adrenergic receptor β2AR gene
AF1	ligand-independent activation function domain 1
AF2	ligand-independent activation function domain 2
AM	alveolar macrophage
AP-1	activator protein 1
BALF	bronchoalveolar lavage fluid
BMI	body mass index
CBP	CREB-binding protein
CCL2	chemokine ligand 2
CD	Crohn's disease
cDNA	complementary DNA
CI	confidence interval
COPD	chronic obstructive pulmonary disease
COX-2	cyclooxygenase-2
CSE	cigarette smoke extract
CVA	canonical variates analysis
CYP1A1	cytochrome P450 1A1
CYP1B1	cytochrome P450 1B1
DBD	DNA binding domain
DL_{CO}	diffusion capacity for carbon monoxide
EGF	epidermal growth factor
EPHX1	microsomal epoxide hydrolase
FEV1	forced expiratory volume at 1 second

FVC	forced vital capacity
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
G-CSF	granulocyte colony stimulating factor
GM-CSF	granulocyte-macrophage colony stimulating factor
GO	gene ontology
GR	glucocorticoid receptor
GRR	genotype relative risk
GST	glutathione S-transferase
GWAS	genome wide association studies
HDAC	histone deacetylase
HMOX-1	heme oxygenase-1
HSP	heat shock protein
HWE	Hardy-Weinberg equilibrium
IBD	inflammatory bowel disease
IFN-γ	interferon-γ
IHC	immunohistochemistry
IL17	interleukin-17
IL-1b	interluekin-1b
IL-23	interleukin-23
IL-6	interleukin-6
IL-8	interleukin-8
iNOS	inducible NO synthase
LBD	ligand-binding domain
LD	linkage disequilibrium
LTB_4	leukotriene B_4
LXR	liver X receptor
mab	monoclonal antibodies
MAF	macrophage activating factor
MCP-1	monocyte chemotactic protein-1
MDMs	MO derived macrophages
MIP3-α	macrophage inflammatory protein 3-α

MKP-1	mitogen-activated protein kinase phosphatase-1
MMP	metalloproteinase
MO	monocytes
NE	neutrophil elastase
NFAT	nuclear factor of activated T cells
NF-κB	nuclear factor κB
NR	nuclear receptors
OR	odds ratio
p/CAF	p300-CBP-associated factor
PBMC	peripheral blood mononuclear cells
PLA2G7	phospholipase A2
PPARE	PPAR response element
PPARγ	peroxisome proliferator-activated receptor gamma
PPIA	peptidyl-prolyl isomerase A/CyclophilinA
PSC	primary sclerosing cholangitis
RA	rheumatoid arthritis
ROS	reactive oxygen species
RPLP0	ribosomal phosphoprotein large P0
RXR	retinoic acid receptors
SAGE	serial analysis of gene expression
SFTPB	surfactant protein B
SIRT1	sirtuin-1
SLE	Systemic lupus erythematosus
SLP1	secretory leukocyte protease inhibitor
SNP	single nucleotide polymorphism
SPP1	osteopontin
STATs	signal transducer and activators of transcription
TF	transcription factor
TGF-α	transforming growth factor-α
TGF-β	transforming growth factor-β
TIMP	tissue inhibitors of MMP

TLDA	TaqMan Low Density Arrays
TNF-α	tumor necrosis factor-α
TSLP	thymic stromal lymphopoietin
TZDs	thiazolidinediones
UC	ulcerative colitis
UPL	probes Universal Probe Library system
VDBP	vitamin D binding protein

2 INTRODUCTION

Chronic inflammatory diseases have shown increasing incidence in the past thirty years, afflicting millions of people in worldwide placing an elevated burden on health-care services in both industrialized and developing countries. Allergic conditions (asthma, eczema), as well as disorders with autoimmune origin, such as rheumatoid arthritis (RA), psoriasis and inflammatory bowel diseases (IBD), chronic obstructive pulmonary disease (COPD) and neurodegenerative disease are part of this group of conditions [1, 2]. The general cause of these conditions is shared, it is an unregulated inflammatory process, which affects specific organ systems or cause systemic inflammation.

Inflammatory response is part of the normal host defense mechanisms, protects from infection, initiates pathogen killing and plays role in tissue repair processes [3]. Interaction of many cell types and production of chemical mediators are involved in the inflammation. Abnormal response to pathogens or external stimuli such as environmental pollutants could lead to an unregulated inflammatory process causing overproduction of cytokines, chemokines oxidants and proteases. The elevated levels of these mediators amplify the inflammatory response and contribute the development of clinical symptoms in genetically susceptible hosts, which suggests that the combination of environmental factors and genetic susceptibility is essential during the development of chronic inflammatory disorders [4].

Sequencing of the human genome allows identification of the genetic variation such as single nucleotide polymorphisms (SNP) and copy number variations. SNPs, which are variations of the DNA sequence that occurs when a single nucleotide in the genome sequence is altered and the frequency of the rare allele >1% in the population, cause about 90% of all human genetic variation. The International HapMap Project, which provides key resource of human genetic variation data, contains allele frequency and the linkage disequilibrium (LD) information (correlation between nearby variants), across several populations for approximately 3.5 million SNP. However, recently the 1000 Genomes Project Consortium has described the allele frequency and local haplotype structure of more than 15 million SNPs, majority of them were not described previously.

Although approximately the 99.5% of human DNA sequences are the same between individuals, the genetic variations can have a major impact on how individuals respond to disease and environmental factors [5-7].

Since genetic predisposition is one of the major causes of chronic inflammatory diseases, it is very important to identify genetic susceptibility loci. This knowledge may lead to the identification of the pathomechanism of the inflammatory process, to improve new effective therapies and better prediction of the development of the disease [8]. During the past decade individual SNP studies and genome wide association studies (GWAS) identified a large number of robust associations between specific chromosomal loci and complex human diseases such as type 2 diabetes and RA [9-11]. Associations were also found between common variant of SNPs and diseases. Since these are multifactorial disorders and many common SNPs were also identified as susceptible factors the prevalence of the combination of risk factors gives better prediction of the risk of the disease, in contrast to monogenic disorders such as cystic fibrosis where a gene mutation lead to the development of the disease [12].

The most studied SNPs are the non-synonymous SNPs that are localized in the protein-coding region, cause amino acid change, which can affect the protein function. These polymorphisms can be associated with the development of the disease or have protective effect. Variation of gene transcription is crucial in mediating disease susceptibility therefore; SNPs localized in the regulatory regions may contribute to disease specific gene expression pattern. Several synonymous SNPs also identified as disease susceptible genetic variants. Although they do not cause amino acid change these variants can affect the mRNA stability or protein folding [13, 14].

Recent development in genomic technologies, including the extensive use of microarray technology provided an opportunity to perform global gene expression analyses. This is a hybridization-based technique, which enables to determine relative mRNA levels of thousands of genes from a sample simultaneously [15]. In the last decade gene expression arrays were developed by standards both for research use and for clinical application, which lead to obtain more reliable data [16]. Microarray experiments allow us to compare the transcriptome of tissue samples or distinct cell types [17, 18]. The comprehensive assessment of gene expression profiling of biological samples using

high-throughput technologies has already led to the identification of disease subtypes, novel genes, biomarkers for disease, as well as the identification of transcriptional networks associated with disease traits (Figure 1).

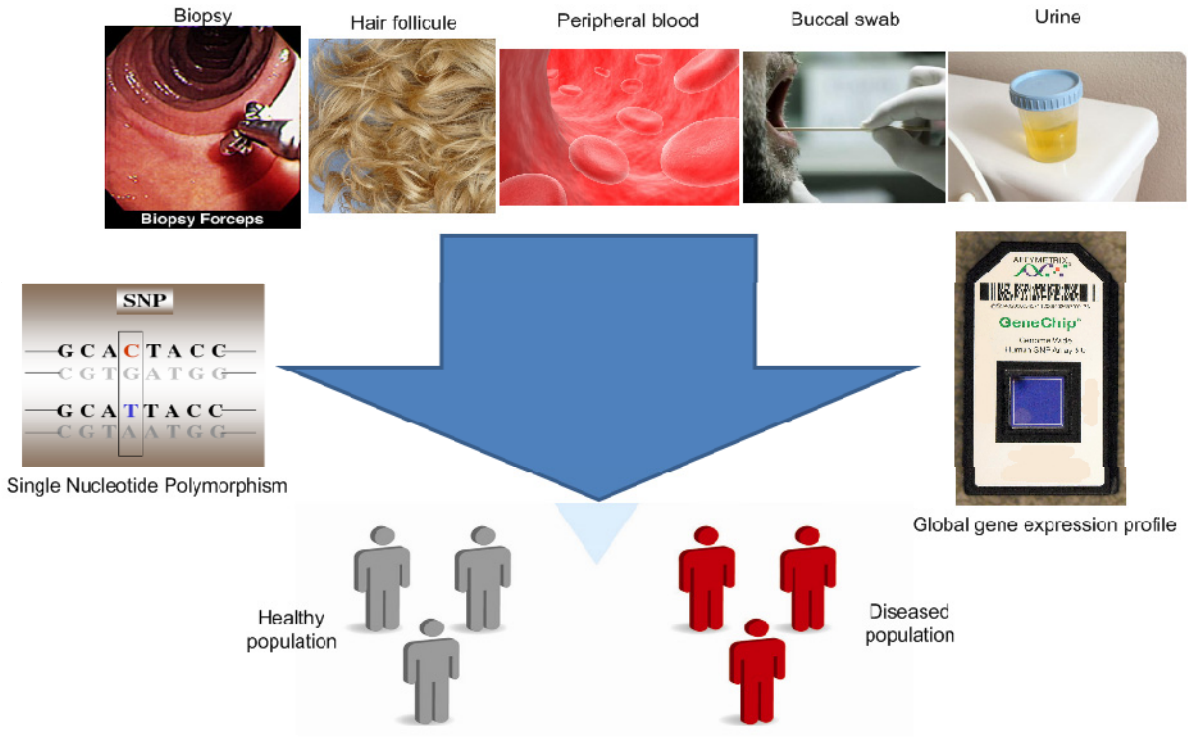


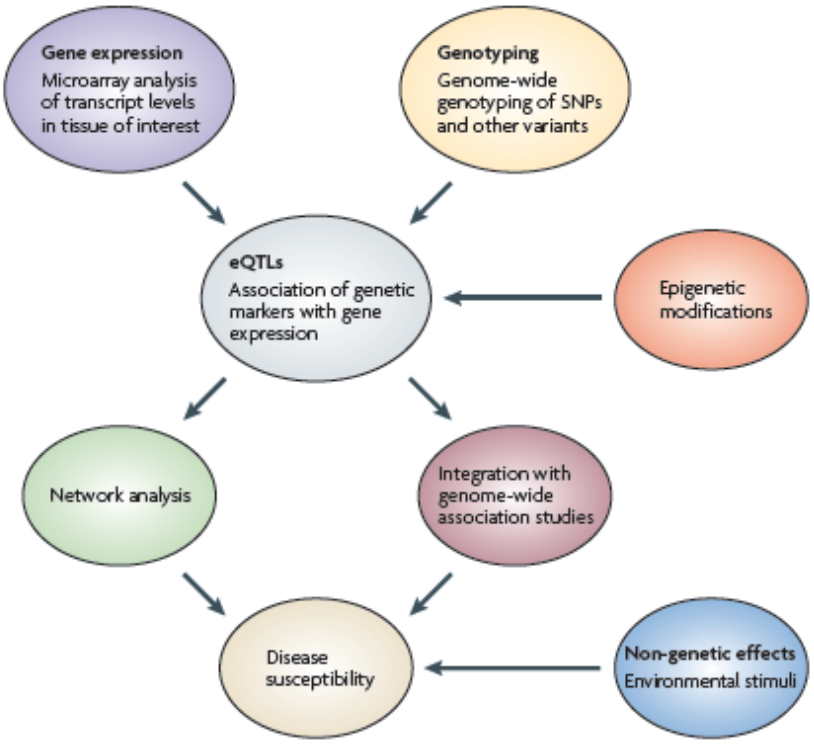
Figure 1. Large-scale genomic approaches

Complex diseases, such as tumors show high heterogeneity at molecular level, which reflects different clinical stages. Although classical histopathological techniques are appropriate for diagnose major forms of cancer but some recent studies showed that global gene expression analysis gave better classification of distinct cancer sub-types.

Large-scale gene expression analysis of peripheral blood B-cells revealed different sub-populations a B-cells in diffuse large B-cell lymphoma, which discriminated different clinical stages of the affected patients [19]. Histologic grade in breast cancer provides clinically important prognostic information. Based on mRNA profiling the histologic grade 2 tumors could be distinguished into two groups with high and low risks of recurrence [20]. Patients with breast cancer having the same stage of the disease can show markedly different response to treatment and overall outcome. Results

of gene expression analysis of breast tumors gave better classification of patients and provided a strategy to select patients for adjuvant therapy [21]. However squamous cell carcinomas are histologically similar, they have distinct gene expression profiles based on their anatomical origin. Gene expression analysis is useful to identify the derivation of lung nodules and enhances treatment planning [22].

Tumor necrosis factor alpha (TNF-α)-blocking agents are used in the therapy of different autoimmune disorders, such as IBD and RA. Because 30% of patients do not respond properly to the treatment, there is an important issue to identify differentiating markers between responders and non-responders. Several studies in RA showed that RNA expression profiling of blood samples is useful to identify marker gene set, which correlated with good clinical response or with therapeutic failure [23].



(Nature Reviews Genetics **10**, 184-194)

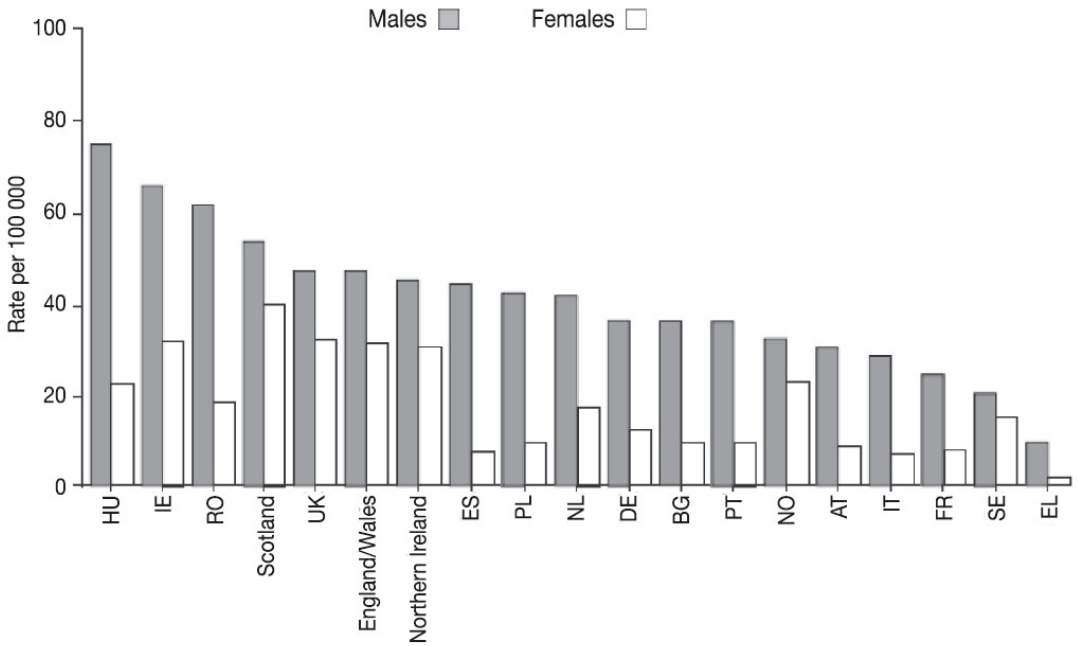
Figure 2. From genome-wide analysis to disease susceptibility.

Identification of genes with altered expression or marker genes could be potential targets for novel and more effective therapy. Integrated functional genomics is an

approach that combines results from multiple genomic analyses or proteomic analysis and functional experiments to identify important genetic signals underlying biological processes. Integration of large scale genomic, proteomic and metabolomic data is critical for investigation and understanding the molecular basis of complex diseases. Using systems biology approaches provided a method, which is the basis of the new paradigm for drug discovery proposed by Schadt and colleagues termed Network Based Drug Discovery (NBDD) [24] and leads on the way toward personalized medicine [25] (Figure 2).

2.1 COPD

COPD is an increasing global health problem, it is predicted to become the third most common cause of death and the fifth most common cause of disability in the world by 2020 [26, 27]. In 2005, chronic respiratory diseases caused more than 4 million deaths, including over 3 million deaths from COPD, worldwide. COPD is one of the main causes of mortality in Europe and Hungary is one of most affected country. Mortality rates for COPD are two or three times higher for males than for females. The age-adjusted rates for men are the highest in Hungary among the European Union and the Central East European countries, while the countries with the highest rates for women (more than 20 per 100 000) are Hungary, Ireland, United Kingdom and Norway, according to data published by the European Respiratory Society [28, 29] (Figure 3).



Sources: Reproduced from European Respiratory Society 2003.

Figure 3. Age-adjusted death rates per 100 000 for COPD for males and females in European countries.

Although in the past few years the pathomechanism of asthma has been examined frequently and new effective therapies were found, in COPD the exact molecular

mechanisms leading to the disease development and effective treatment are not known yet [30, 31]. However, COPD and asthma involve chronic inflammation of the respiratory tract, but there are many differences in the site of inflammation and in the involved inflammatory cell types [32-35]. Asthma is characterized by increased numbers of eosinophils and CD4+ T lymphocytes. The inflammation is affects all of the airways and does not involve the lung parenchyma. Airway hyperresponsiveness is linked to eosinophilic inflammation, although its mechanism is uncertain. In contrast to COPD where corticosteroids do not appear to have effect on the inflammation, inhaled corticosteroids suppress the eosinophilic inflammation in asthmatic patients. This suggests that different patterns of mediators play role in the two diseases and in COPD some patients develop systemic effect of the disease [36-38].

COPD is characterized by slowly progressive development of airflow limitation, which is mostly irreversible in contrast to asthma where airway obstruction is usually reversible. The airflow limitation is associated with an abnormal inflammatory response to noxious particles or gases in the lungs [39, 40]. COPD is accompanied by emphysema, chronic bronchitis with airway obstruction and small airway disease, these are distinct phenotypes of COPD, but most patients show a combination of these phenotypes [41]. Emphysema is characterized by a Th1 type inflammation, destruction of lung parenchyma and enlargement of peripheral airspaces, including respiratory bronchioles, alveolar ducts and alveoli and loss of lung elasticity. The inflammation in chronic obstructive bronchitis affects the large airways with goblet cell hyperplasia, mucus hypersecretion and chronic sputum production. Small airway disease mainly affects the bronchioles. Based on the lung function four severity classes can be classified in COPD (GOLD Guidelines) [42-46].

There are several risk factors of COPD; life and work circumstances, environmental pollution and aging, but cigarette smoke is the major risk factor for development of COPD. Cigarette smoke contains an estimated 10^{15}-10^{17} free oxidant radicals and more than 4500 reactive chemical compound, and it is the primary cause of COPD including emphysema and chronic bronchitis. In susceptible smokers the lung functions, forced vital capacity (FVC) and forced expiratory volume at 1 second (FEV1), rate decline are faster than in healthy smokers and in non-smokers [47-49]. Cigarette

smoke and other irritants induce inflammation in lung tissue, which causes activation of epithelial cells and abnormal migration of different types of inflammatory cells. However, only 15-20% of smokers develop COPD suggesting an additional risk factor the genetic susceptibility also important in the pathogenesis of COPD [42, 50, 51].

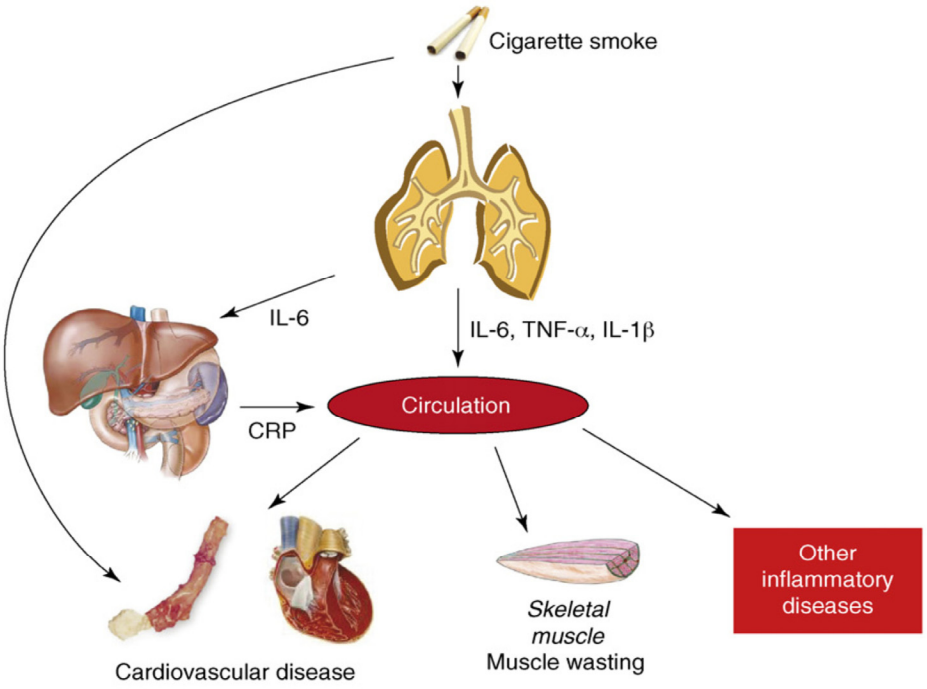
2.1.1 SYSTEMIC CO-MORBIDITIES OF COPD

Beside the manifestation of the disease in the lungs, many patients with severe COPD develop several systemic co-morbidities and the presence of airflow limitation greatly increases the chance of development of lung cancer. Elevated levels of circulating cytokines, chemokines and acute phase proteins cause systemic inflammation in patients with severe COPD. The best recognized systemic manifestations include coronary artery disease and atherosclerosis, loss and dysfunction of skeletal muscle, anemia, osteoporosis and lung cancer [52] (Figure 4).

Since there is anatomical and functional relation between the lungs and the heart, any dysfunction that impacts in one of the organs could cause malfunction of the other. There is a strong correlation between impaired lung function (FEV1) and cardiovascular morbidity and mortality and this is independent of age, sex and smoking history [53].

Systemic inflammation is an important factor in the pathogenesis of weight loss and wasting muscle mass. Enhanced oxidative stress with increased level of reactive oxygen species (ROS) and reduced antioxidant capacity contribute to the progression of skeletal muscle dysfunction in COPD [54]. ROS can increase muscle cell apoptosis and muscle proteolysis and inhibit muscle-specific protein expression [55].

The increased inflammation and oxidative stress increase the prevalence of lung cancer in COPD patients. Pro-inflammatory cytokines may promote tumor angiogenesis, which accelerates cell growth and metastasis. Patients with COPD develop lung cancer with 3-4 times more compared to smokers with normal lung function and lung cancer is a common cause of death among patients with severe COPD [56, 57].

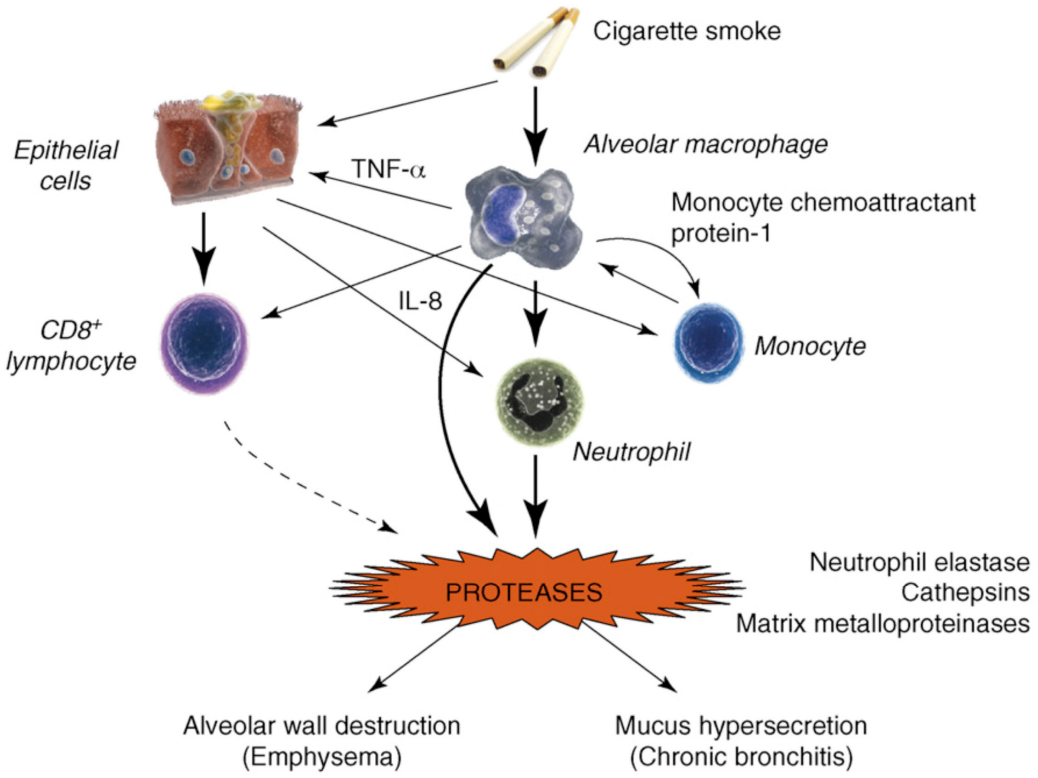


TRENDS in Pharmacological Sciences, Vol.28 No.10

Figure 4. Systemic co-morbidities of COPD

2.1.1 INFLAMMATORY CELLS IN COPD

As COPD is complex inflammatory disease, several types of inflammatory cells are involved in the development of the disease. Increased number of implicated cell types such as macrophages, neutrophils, dendritic cells (DC), T-lymphocytes and B-lymphocytes are observed in alveoli and small airways [33, 38, 58-60] (Figure 5).



TRENDS in Pharmacological Sciences, Vol.28 No.10

Figure 5. Inflammatory cells in COPD

Cigarette smoke and other inhaled irritants is the major cause of the inflammation in COPD. This inflammatory process is characterized by increased numbers of neutrophils, AMs and CD8+ lymphocytes. AMs play crucial role in orchestrating the inflammation through the release of inflammatory cytokines and proteases.

2.1.1.1 Neutrophils

In early stage of the inflammation neutrophils are the dominant inflammatory cells. Increased numbers of activated neutrophils were found in sputum and bronchoalveolar lavage fluid (BALF) of patients with COPD [61]. These cells secrete different types of proteases such as serine proteases including neutrophil elastase (NE) and cathepsin G as well as matrix metalloproteinases (MMP)-8 and MMP-9, which cause alveolar destruction and are potent mucus stimulant. Neutrophil recruitment to the airways and parenchyma and their migration into the respiratory tract is controlled by neutrophil chemotactic factors including leukotriene B_4 (LTB_4), interleukin-8 (IL-8) and

related CXC chemokines, which are increased in COPD [62, 63]. The source of these mediators could be the alveolar macrophages (AM) and lung epithelial cells, but the neutrophils itself produce large amount of IL-8. Cytokines such as granulocyte-macrophage colony stimulating factor (GM-CSF) cause increased survival rate in the respiratory tract [64]. Smoking has a direct stimulatory effect on granulocyte recruitment from the bone marrow, possibly mediated by GM-CSF and granulocyte colony stimulating factor (G-CSF) and may also increase neutrophil retention in the lung [65]. Activated neutrophils show an increase in the respiratory burst response, correlating with the rate of airflow limitation and rapid decline of FEV1 [66, 67].

2.1.1.2 Lymphocytes

T-cells also show increased number in lung parenchyma and peripheral and central airways of patients with COPD. In the population of T-cells the ratio of $CD4^+$ / $CD8^+$ cells are reversed; however the absolute number of $CD4^+$ T cells are also increased [48, 68-71]. The majority of T-cells belong to the Th1 (interferon-γ producing) subtype in COPD airways, releasing perforins, granzymes and tumor necrosis factor-α (TNF-α), associated with apoptosis and necrosis of lung cells. Th2 (interleukin-4 producing) subtypes of T-cells are accumulated around mucus-secreting glands in patients with chronic bronchitis [72, 73]. The mechanisms of T-cells accumulation in the airways and lungs of patients with COPD are not yet understood. Their homing to the lungs must depend on selective chemotaxis. The role of T-cells in the pathophysiology of COPD is also not yet known [74]. B-cells may produce altered antibodies in response to cigarette smoke in patients with COPD, however the pathways for antibody production are not known [75].

2.1.1.3 Dendritic Cells

DCs have central role in the initiation of the innate and adaptive immune response and are essential for inducing activation and differentiation of naïve and also effector $CD4^+$ and $CD8^+$ T-cells in response to inhaled antigen [76]. Various lung compartments contain DCs, but the lineage or origin of these cells has been poorly defined [77]. In mouse model, cigarette smoke exposure increased the numbers of $CD11c^+$ DCs were

observed, which is explained by elevated levels of monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein 3-α (MIP3α) [78, 79]. Lung DCs produce high level of MMP-12, contributing to tissue damage. This mechanism may be reliable in COPD patients, as increased numbers of DCs were found in the epithelium of small airways, correlating with high levels of MIP3α in induced sputum [80]. Airway smooth muscle cells of COPD were found to produce more DC maturation factor thymic stromal lymphopoietin (TSLP) [81]. Beside these findings, the exact role of DCs initiating airway inflammation and emphysema is still largely unknown.

2.1.1.4 Epithelial cells

Cigarette smoke activated airway and alveolar epithelial cells are important source of inflammatory mediators producing TNF-α, IL-8, interluekin-1β (IL-1β) and GM-CSF [82, 83]. In small airways, they express transforming growth factor-β (TGF-β), which is an inducer of local fibrosis [84]. Epithelial cells are important in defense of the airways, secreting defensins and other cationic peptides with antimicrobial effects and are part of the innate defense systems [85]. Increased proliferation of airway epithelial cells causes squamous metaplasia, which is very often in chronic bronchitis and COPD. This proliferation is induced by elevated expression of epithelial growth factors and may contribute an increased risk of bronchial carcinoma [86, 87].

2.1.1.5 Monocytes/Macrophages

Alveolar macrophages (AM) seem to play a central role in the pathogenesis of COPD and the maintenance of the inflammatory process [88, 89]. AMs are the major host defense cells in the lower airspace. Cigarette smokes exposure causes more than fivefold increase in total cells in BALF, 95-98% of these cells are macrophages [74]. The population of AMs recovered from BALF is phenotypically heterogeneous. A part of them have normal appearance, while others enlarged and are full with engulfed particles and there is a third subset with smaller size than the normal pulmonary macrophages, may be immature cells [90]. The precursors of AMs are monocytes (MO), which are released from bone marrow and are homing to the lungs in response to cigarette smoke, but higher rate of MO recruitment from peripheral blood was also observed in response to

monocyte-selective chemokines such as MCP-1 and lymphocyte chemotactic factors released by T-cells [63, 91]. Macrophages have a long life span, up to several months under normal condition. In smokers AMs may exist for more than 2 years before clearance and cell death [92]. There is correlation between macrophage numbers and disease severity. Macrophages are localized to alveolar wall destruction in patients with emphysema [68, 93].

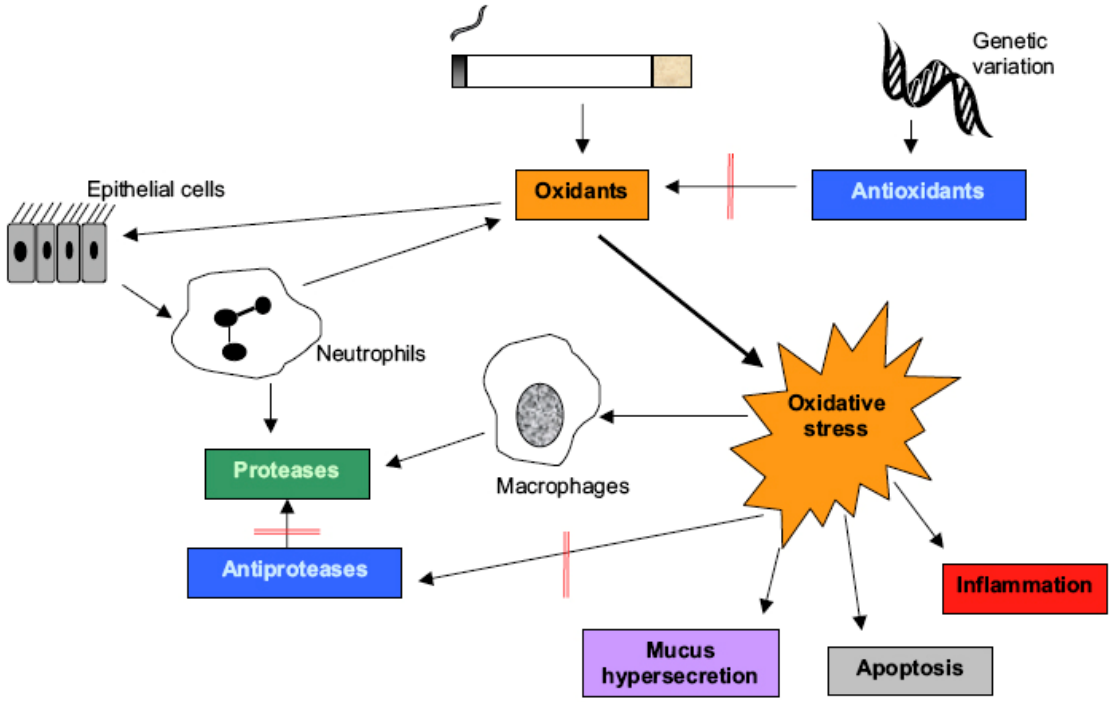
AMs secrete large number of inflammatory mediators including chemokines, cytokines, growth factors, lipid mediators and reactive oxygen and nitrogen species. AMs can be activated by cigarette smoke, pro-inflammatory cytokines and immune stimuli. In patients with COPD AMs show increased release of inflammatory mediators compared to normal smokers. They secrete higher level of IL-8 and IL-1β after cigarette smoke activation, which lead to enhanced recruitment of neutrophils in COPD [94, 95]. Releasing other CXC type chemokines such as CXCL-10 and CXCL-9, AMs play key role in the recruitment T lymphocytes to the lungs [73]. The production of interferon-γ (IFN-γ) and elevated level of TNF-α result the maintenance of the chronic inflammatory cycle. In chronic bronchitis macrophages release TGF-β, which may contribute to the fibrosis in small airways. They also produce transforming growth factor-α (TGF-α), an endogenous activator of epidermal growth factor (EGF) receptors that have key role in regulation of mucus hypersecertion [33].

Many types of elastolytic enzymes, such as MMP-2, MMP-9, MMP-12 and cathepsins are secreted by AMs [96-98]. The MMPs are belonging to family of Zn^{2+}-dependent and Ca^{2+}-dependent proteinases, degrading the extracellular matrix proteins. They play an important role during the development of healthy tissue as well as remodeling of damaged tissue. However increased expression of MMPs and decreased expression their inhibitors such as tissue inhibitors of MMPs (TIMPs) were reported in patients with COPD and may the major cause of emphysema. MMP-9 is strongly expressed in emphysematous lung mostly by AMs [98-100]. However, there are controversial data about the role MMP-12 in the pathogenesis of COPD, elevated macrophage derived levels of MMP-12 is described in smokers' lungs and it appears to play an important role in development of cigarette smoke-induced emphysema [88, 101]. MMP-$12^{-/-}$ mice did not develop emphysema in response to long-term cigarette smoke

exposure in contrast to MMP-$12^{+/+}$ mice, confirming this role of the enzyme [102]. AMs also can release neutrophil elastase although they do not synthesize it. It derives from neutrophils after macrophages internalized them, suggesting AMs may represent a reservoir for neutrophil elastase [103, 104]. Cigarette smoke activated macrophages produce large amount of unstable free oxidative radicals, such as superoxide anion, peroxynitrite and hydroxyl radicals. Formation of these reactive particles leads to a series of chain reactions, which yield tissue destruction [105].

2.1.2 GENETIC RISK FACTORS OF COPD

Development of complex genetic diseases, such as COPD is associated with the interaction of environmental factors and genetic susceptibility. Genetic association and genome-wide studies have identified several genes and their SNPs that might be involved in the pathogenesis of COPD. Since the proteases-antiproteases and oxidants-antioxidants balance are crucial in COPD, most of the associated genes functions correlate with these two pathways [34]. However SNPs of other candidate genes, which are part of the inflammatory process or play role in immune response, have been also found to show association with COPD (Figure 6). SNPs of candidate genes, which show strong association with the disease, could be potential biomarkers, predictors of the disease or targets of new therapies [42, 106-108].



Respiratory Research 2006, **7**:130

Figure 6. Pathways of COPD associated gene polymorphisms.

Cigarette smoke contains irritant and oxidant particles, which activate neutrophils and macrophages and induce oxidative stress, inflammation and secretion of proteases. The balance of oxidants/antioxidants and proteases/antiproteases is important in the severity of COPD. SNPs of susceptible genes, which are related to these pathway could modulate positively or negatively the pathomechanism of the disease.

2.1.2.1 Proteases-antiproteases

Alpha$_1$-antitrypsin (α_1-AT) is an acute phase protein, which provides major defense against NE. α_1-AT is synthesized predominantly in the liver, but AMs also secrete it. It is a highly polymorphic protein; more than 70 variants have been identified. The most common gene variants are the M, S, and Z alleles. Patients with the MM genotype are defined as normal and have the highest α_1-AT levels. MS and MZ genotypes lower levels of the normal and the rare SZ heterozygotes show more reduced levels of α_1-AT. ZZ homozygote variant associated with α_1-AT deficiency and higher

risk of development of emphysema. Individuals with ZZ genotype have a clearly accelerated rate of decline in lung function [109-112].

Several studies in animals and humans showed that MMP-1, MMP-12 and MMP-9 are important in airway inflammation and development of emphysema and show elevated levels in smokers [113]. SNPs of MMPs genes were examined in genetic association studies and found that promoter polymorphisms in MMP genes alter gene expression [114]. Haplotype variants consisting of alleles from MMP-1 G-1607 GG and MMP-12 Asn357Ser SNPs were associated with rate of decline of lung function which is suggest that polymorphisms of MMP-1 and MMP-12 genes are causative factors in smoking-related lung injury [115]. A promoter variant of MMP-9 (C-1562T) reported to be increased its activity and linked to COPD in Chinese Japanese and Korean population [116-118].

SERPINE2 gene, a member of serpin peptidase inhibitors, was identified as having potential role in COPD [119]. It is known to be an inhibitor of trypsin-like serine proteases, but not NE and might have a role in the protease-antiprotease pathway [120]. Multiple SNPs of this gene were examined in the Boston early-onset COPD cohort and in patients from the NETT cohort with several being significantly associated [121]. However case-control studies could not replicate these associations with COPD in European patients, which questioned the validity of some the results found in the original study [122].

2.1.2.2 Antioxidants and xenobiotic metabolizing enzymes

Cigarette smoking causes high level of oxidative stress and accumulation of toxic substances in the lungs. Genetic variation of enzymes in the metabolism and detoxification of noxious particles such as hydrocarbons, epoxides and oxidants are important determinants of host response [105, 123, 124].

Cytochrome P4501A1 (CYP1A1) is a member of cytochrome P450 family is capable metabolizing xenobiotic compounds and may play role in the activation of procarcinogens. SNP of CYP1A1 in the exon 7 causes amino acid substitution (Ile462Val) and the Val462 allele, which alters higher enzyme activity, was associated with centriacinar emphysema in patients with lung cancer [125, 126].

Microsomal epoxide hydrolase (EPHX1) is an enzyme associated with the metabolism and detoxification of xenobiotic chemicals; it plays an important role in the general oxidative defense of lung. Several polymorphisms are known in EPHX1 including two relatively common SNPs, the exon 3 Tyr113His (rs1051740) and exon 4 His139Arg (rs2234922) variants. These two variants of alleles have been suggested to be associated with altered EPHX1 enzyme activity. Substitution of Tyr113 for His decreases (slow allele), whereas substitution of His139 for Arg (fast allele), increases EPHX1 activity [127]. Since EPHX1 is involved in the detoxification of epoxide intermediates in tobacco smoke, any change in the rate of conversion of these highly reactive compounds could affect an individual's ability to cope with the toxic effect of cigarette smoke. Several studies found the slow metabolizing form of EPHX1 to be associated with an increased risk for COPD [128-133].

2.1.2.3 Inflammatory mediators

TGF-β regulates extra-cellular matrix production, tissue repair, and cell growth and differentiation, and have role in immune response. In the Boston early-onset COPD cohort a linkage analysis showed association between TGF-β1 gene and FEV1 [134]. This gene was also examined in the NETT cohort and three SNPs of it were significantly associated with severe COPD [130]. The association of two of these SNPs was replicated in other study and showed both of them have effect on TGF-β levels. The first (C→T change at position -509) is located in the promoter region; causing enhanced promoter activity increases the TGF-β1 level. The second that leads amino acid change (Leu→Pro) at position 613 elevates the production of TGF-β1. In case-control study the Pro allele was found to less common in patients with COPD relative to healthy smokers, suggesting a protective role of this allele [135, 136].

TNF-α is key factor to initiate and mediate the inflammation in both the respiratory tract and systemic features of COPD. Several polymorphisms of TNF-α were examined in case-control studies but the most studied is a promoter variant (-308G>A), which increases the production of TNF-α through directly affect the gene transcription. The -308G>A SNP linked to increased airway inflammation in Taiwanese patients with

chronic bronchitis and also linked to airflow obstruction in subjects without chronic bronchitis and severity of emphysema in a Japanese study. However these findings could not be validated in Caucasians. Variation in genotype frequencies between races might be a possible explanation of these controversial findings [128, 133, 137].

Peroxisome proliferator-activated receptor gamma (PPARγ) is involved in the regulation of inflammatory signaling pathways of various autoimmune disorders, therefore polymorphisms of PPARγ could be implicated in the susceptibility to these diseases. Association of PPARγ SNP with type 2 diabetes was reported in several studies. The minor allele of Pro12Ala, which causes lower binding affinity of PPARγ protein to PPRE, was associated with lower body mass index, improved insulin sensitivity and decreased risk of type 2 diabetes [138, 139]. Controversial data were found in association to risk of colorectal cancer. The Ala allele was also associated with reduced risk to develop colorectal cancer while higher frequencies of the minor allele of the synonymous His447His (C>T) polymorphism was observed in colon cancer patients. In a haplotype analysis study the combination of Ala allele and minor allele of His447His associated with reduction risk of colorectal adenomas. Three SNPs of PPARγ were examined in asthma (Pro12Ala, His447His, and C-681G) [140, 141]. The combination of major alleles of Pro12Ala and His447His was associated with increased risk for asthma exacerbations; in another study this haplotype combination was associated with aspirin sensitivity in asthmatic patients [142-144].

2.1.2.4 Mediators of physiological processes in the respiratory tract

β_2-agonists are the most common bronchodilators used to treat airflow limitations in asthma and COPD [145]. They are act through β_2-adrenergic receptor (β2AR) gene (ADRB2). The β2AR mediates bronchodilation, mucociliary clearence, vascular endothelial permeability and anti-inflammatory responses in the airways. Several clinical studies have shown the genetic contribution of the Arg16Gly polymorphism to short-term bronchodilator response to β_2-agonists in patients with asthma [146]. In a Japanese study the Arg16Gly polymorphism have been found to be have a significant physiologic role in regulating responses to exogenous β2AR agonists in patients with COPD [145]. Two

SNPs of ADRB2 were associated with emphysema severity airway wall thickness phenotypes in the NETT cohort [147].

The surfactant proteins contribute to regulation of surface tension in the alveoli and also have role in the host defence and control of inflammation. A SNP in surfactant protein B (SFTPB), which causes amino acid change (Thr131Ile), has been associated with COPD in Boston Early-onset COPD cohort and in a case-control study in Mexico [129, 130].

2.1.3 GENE EXPRESSION STUDIES IN COPD

In complex diseases it is also very important to study comprehensive changes between normal and unhealthy state. Several recent studies attempt to identify novel pathways or gene sets involved in the pathogenesis of COPD using gene expression profiling analyses. There was only a minimal overlap between the differentially expressed genes among the different datasets. These differences could be derived from several factors, such as differences in analysis, sample collection and size, disease severity, examined tissue or cell components and not the same expression platform. Beyond the variability of the studies, these findings have presented useful information to the pathogenesis of COPD [148].

Global gene expression pattern was examined of normal and emphysematous lung tissue, using Affymetrix microarray. In this study genes were identified which can distinguish normal lung tissue from severely emphysematous lung as well as genes that correlate with lung function parameters such as FEV1 and diffusion capacity for carbon monoxide (DL_{CO}) and associated with systemic effect of emphysema, like lower body mass index (BMI) [149].

Lung tissue samples of GOLD-2 smokers and GOLD-0 smokers were compared using serial analysis of gene expression (SAGE) and microarrays, to better understand the molecular basis of COPD. Several classes of genes were found, many of which have not been previously associated with COPD [150, 151].

The effect of cigarette smoking on gene expression of airway epithelial cells was compared between healthy smokers and healthy nonsmokers and also between healthy

smokers and smokers with COPD. Pathway analysis of the differentially expressed genes revealed relevant functions to the pathogenesis of COPD [152, 153].

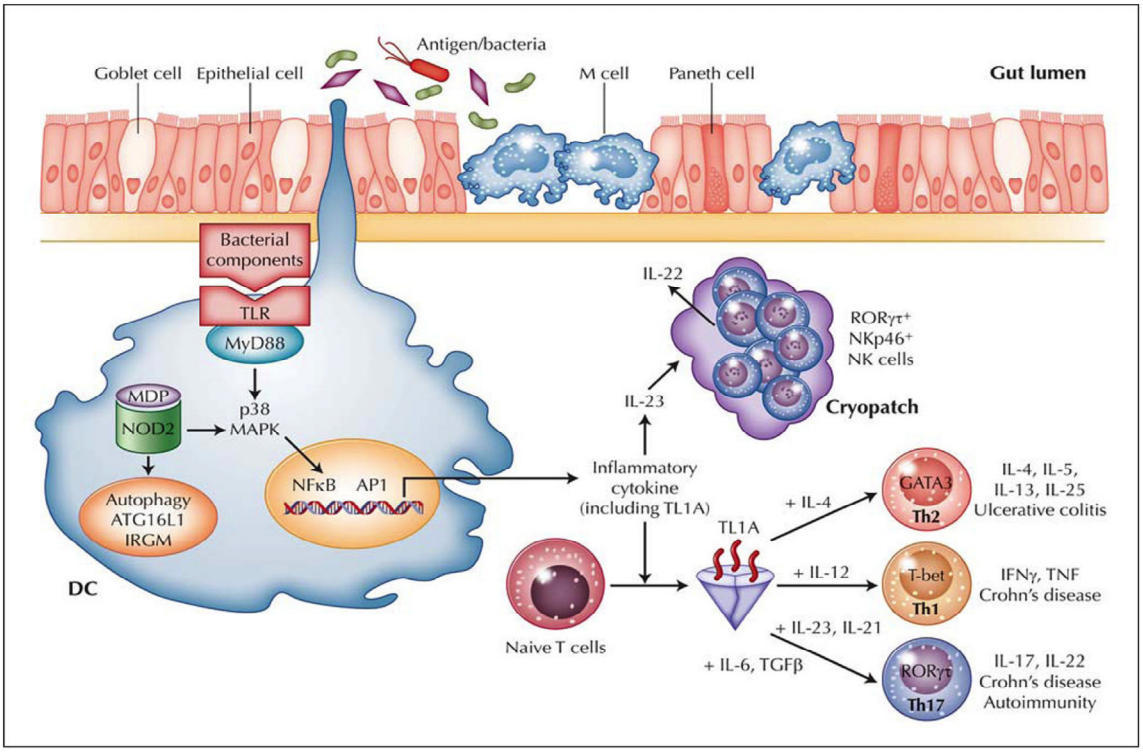
The smoking effect was also examined gene expression profiling of AMs, which were obtained from BAL and provided novel candidate genes for susceptibility to smoking-related COPD and other lung diseases. CSE induced cytokine production was characterized using MO derived macrophages (MDMs). To characterize the smoking related gene expression changes AMs were obtained from transgenic mouse model of emphysema. Although the degree of similarity between human smokers and the animal model was much more restricted and there are inevitable limitations in comparing the expression data between species [154-156].

2.2 IBD

IBD is a chronic inflammatory disorder with autoimmune origin affecting the gastrointestinal tract. It is clinically classified into two major phenotypes: ulcerative colitis (UC) and Crohn's disease (CD). In recent years CD and UC have shows increasing frequency, imposing a significant burden on the healthcare system. Both CD and UC primarily are disease of young adults, incidence occurs between 15 snd 30 years of age. Incidence and prevalence of IBD vary significantly depend on geographical and ethnic background, there is an increased risk for developing IBD in urban areas with a higher socioeconomic class in the developed countries compared with rural areas or less developed countries. Family aggregation has been recognized, first-degree relatives of the affected patients have five fold greater relative risk for developing IBD and the inheritable risk seems to be stronger in CD compared to UC.

Although the etiology of these diseases still remains unknown, the cause of the diseases is better understood, a dysregulated mucosal immune response to the normal intestinal microflora in a genetically susceptible host is involved in the pathogenesis [157]. The normal epithelium provides an effective barrier against luminal agents, defending the integrity of the mucosal barrier is essential. The integrity of the barrier could be compromised by a decreased reparative response to injury, exogenous agents or genetic determinants. The stimulation of the mucosal immune system by the products of commensal bacteria or antigens from dietary sources may contribute to a chronic

recurrent intestinal inflammation in the lumen. The penetration of bacterial products through the injured mucosal barrier leads to their direct interaction with immune cells, especially DCs and T-cells, promotes a classical adaptive immune response. Alternatively, bacterial products could stimulate the receptors of the innate immune system on the surface of the epithelium inducing the cytokine and chemokine production of epithelial cells that lead to recruitment and activation of mucosal immune cells [158]. Migration of innate immune cells such as neutrophils, macrophages and DCs depends on the expression of cytokines and chemokines. These cells generate ROS that are the effectors of inflammation, tissue injury and increase the permeability of the epithelial barrier (Figure 7).



(Curr Gastroenterol Rep. 2009 December ; 11(6): 473–480.)

Figure 7. Mucosal immune response in IBD

Bacterial compartments activate DCs, which activates naïve T-cells by producing inflammatory cytokines. UC seems to be predominately Th2-mediated, whereas CD is a predominately Th1- and Th17-mediated process

2.2.1 PHENOTYPES OF IBD

UC is characterized by diffuse mucosal inflammation, which extends proximally from the rectum to a varying degree and presence of a significant number of neutrophils within the lamina propria and the crypts, where cause formation of micro-abscesses. The severe inflammatory process with production of inflammatory mediators leads the development of extensive superficial mucosal ulceration. In UC the immune response is mediated by Th2 type T-cells and associated with interleukin-4, -5 and -10 [159]. While in case of CD may be patchy and segmental, the inflammation is usually transmural. Any site of the gastrointestinal tract could be affected, but the involvement of the terminal ileum is the most common and the earliest mucosal lesions often appear over Peyer's patches. It is characterized by aggregation of macrophages that form non-caseating granulomas [158, 160]. It is suggested that the immune response in CD is associated with cytokines produced by Th1 type T-cells, such as INF-γ, TNF-α and interleukin-12 (Figure 7).

This pathophysiological concept for IBD is rapidly changing and a third type of immunologic response was described recently. This is the interleukin-23 (IL-23)/interleukin-17 (IL17) pathway, which is induced by IL-23, leading generation of IL-17, interleukin-6 (IL-6) and TNF-α producing subtype of T-cells, Th17 cells [161]. Th17 pathway is critical for the development of inflammation in the majority of animal models of colitis and both IL-23 and IL-17 show elevated expression levels in inflammatory lesions in patients with CD [162-164].

2.2.2 GENETIC FACTORS OF IBD

Several studies indicate that genetic factors appear to predispose some individuals to the development of IBD [165-168]. Recent single locus genetic association and GWAS studies identified several new susceptibility genes and chromosomal loci that are associated with CD or UC such as NOD2/CARD15, IL23R or ATG16L1 [165-168].

NOD2/CARD15 is localized on pericentromeric region of chromosome 16, that chromosomal region was identified as a CD susceptibility locus and designated IBD1 locus. NOD2/CARD15 is an important receptor for intracellular recognition of bacterial

products such as lipopolysaccharid and peptidogylcan and has a role in signaling of innate defense response in the host. It is expressed in monocytes and activates nuclear factor κB (NF-κB). NOD2/CARD15 has been conclusively associated with CD.

IL-23 is a member of the IL-12 family cytokines produced by activated DCs. This cytokine promotes Th17 cell responses and orchestrate additional innate and T cell-mediated inflammatory pathways. IL-23 pathway might cause an aberrant immune response to microbial encounter and showed that depletion of IL-23 was associated with decreased pro-inflammatory responses in the intestine. The association of several SNPs of the IL23R gene with CD and UC confirmed the critical role of IL-23 pathway in IBD pathogenesis. The Arg381Gln polymorphism confers a strong protective affect against CD and modestly associated with UC.

ATG16L1 is new CD susceptibility gene, which plays role in autophagy. Autophagy is important for cellular homeostatic functions, including nutrient and energy generation, structural remodeling, degradation of damaged or long-lived cytoplasmic components, and protection of invading microorganisms. It is an important part of the innate immune armamentarium and links to adaptive immunity by traffic of antigens for immune recognition. Mutations affecting factors, which are involved in autophagy process may lead immune dysregulation seen in IBD [169-171].

2.2.2.1 Role of PPARγ in IBD

PPARγ is strongly expressed in the colon, mostly by the epithelial cells indicating that it may have an important function in the normal and pathological processes of the colon [172-174]. Animal experiments also provided support for a role in PPARγ in the inflammation of the intestine. Rosiglitazone treatment attenuated the symptoms of induced colitis in a mouse model. Genetic evidence for the protective effect of PPARγ was provided by showing that PPARγ +/- animals were more susceptible to colitis than wild type ones [175-177].

5-aminosalicylic acid (5-ASA), an anti-inflammatory agent, that is widely used to treat IBD, is a PPARγ ligand as well [178, 179]. Rosiglitazone, another PPARγ activator, was tested in clinical trials and was found to be effective in treatment of UC [180]. However, the efficiency of the treatment varied between patients suggesting that

differences in the genetic background of patients, such as single nucleotide polymorphisms of PPARγ could play a role in pathogenesis of IBD [181]. Despite of its suggested role as a repressor of inflammatory responses, very few studies have assessed so far the association between the known polymorphisms of PPARγ and IBD [182-187].

2.3 Transcriptional regulation of inflammation

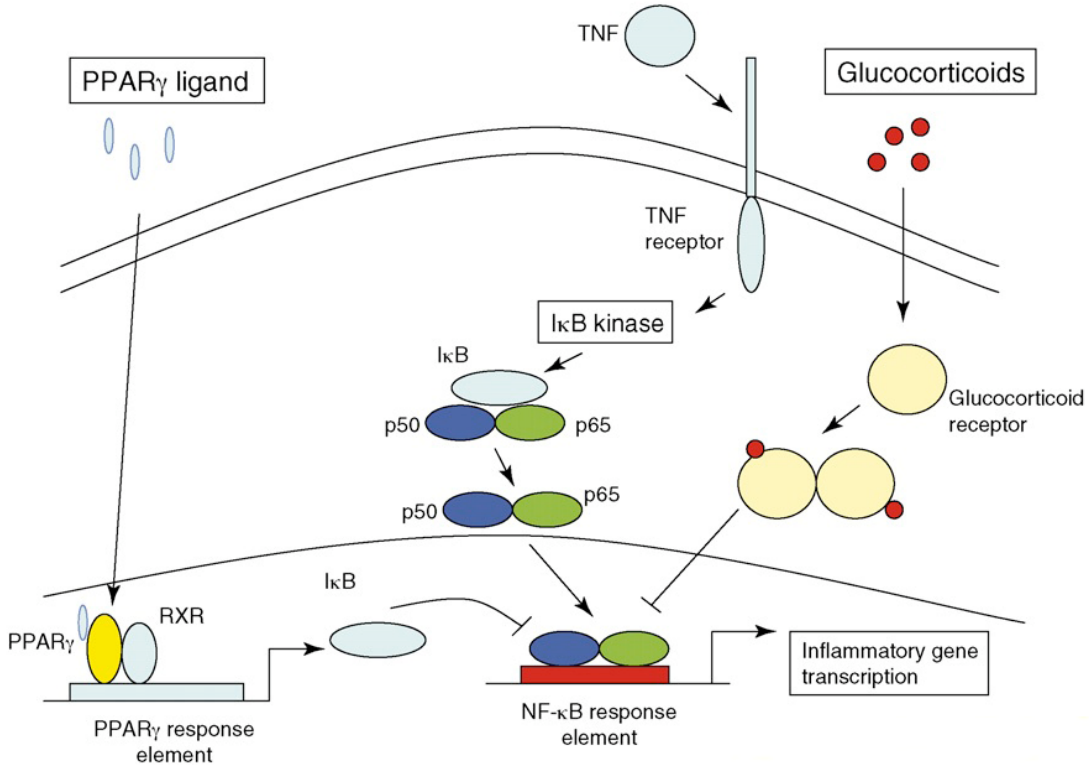
Although inflammation is beneficial in the defense response of the host against pathogens and external stimuli, can become unchecked leading to the pathogenesis of common chronic inflammatory diseases such as RA, type 2 diabetes, multiple sclerosis and COPD. Complex network of signaling mediators are involved in this process, many of them are under the transcriptional control of two transcription factors (TF); nuclear factor κB (NF-κB) and activator protein 1 (AP-1) [188]. NF-κB is a potential therapeutic target; inhibition the activity of this TF can reduce atherosclerosis and type 2 diabetes [189, 190].

Another group of TF, the superfamily of nuclear receptors (NR) is also involved in the regulation of the inflammatory mediators. Several members of NRs have role in the negative regulation of inflammatory response such as glucocorticoid receptor (GR) and PPARγ [191, 192].

2.3.1 NF-κB

Expression of proinflammatory cytokines such as TNF-α and IL-1β is increased in COPD. These mediators amplify the inflammation through activation of NF-κB, which is the key TF of inflammatory genes [51]. Functionally NF-κB is a heterodimer of p50 and p65 proteins [188]. In resting cells this heterodimer located in the cytoplasm in inactive form, bound by inhibitor protein complex of IκB (IκBα and IκBβ). Upon activation of cells such as TNF-α binding, IκB is phosphorylated by protein kinases. This phosphorylation is signal for polyubiquitination and degradation of IκB, which allows a rapid translocation of NF-κB to the nucleus, where it binds to promoters of inflammatory genes, such as TNF-α, IL-8, and GM-CSF and activates transcription of them [193] (Figure 8).

Acetylation and deacetylation also regulate the transcriptional activity of NF-κB. NF-κB interact coactivator molecules, such as CREB-binding protein (CBP)-, p300- and p300-CBP-associated factor (p/CAF), which have acetylase activity. P300/CBP has role in the acetylation of p50 and p65 subunits, which leads to selective gene induction [194]. Acetylated p65 has impaired association with IκBα and enhances the DNA binding affinity of NF-κB complex and preferential binding to the NF-κB binding site in the IL-8 promoter rather than GM-CSF promoter [195]. While acetylation of p50 subunit, which does not have transactivating domain, correlates with increased binding to cyclooxygenase-2 (COX-2) and inducible NO synthase (iNOS) promoters and increases the recruitment of p300 to these promoters [196, 197]. Conversely, members of histone deacetylase (HDAC) family HDAC3 and sirtuin-1 (SIRT1) can inhibit the NF-κB mediated gene transcription through deacetylation of p65 subunit [198]. However, high level of oxidative stress reduces the HDAC activity in COPD [199, 200].



Current Opinion in Pharmacology 2008, 8:275–279

Figure 8. Regulation of inflammatory genes by nuclear receptors

NF-κB is a common TF for proinflammatory genes. NRs, such as PPARγ and GR have role in the negative regulation of inflammatory response through inhibiting the NF-κB function. PPARγ/RXR heterodimer induce the transcription of IκB, inhibitor protein of NF-κB. Ligand activated GR homodimer is translocated to the nucleus, bind to NF-κB and inhibit inflammatory gene transcription.

2.3.2 AP-1

AP-1 is the other important pro-inflammatory TF, consists of homodimers and/or heterodimers of the c-Jun/c-Fos family of proteins [201]. Promoters of many inflammatory genes contain biding sites for both AP-1 and NF-κB, suggesting the possibility of synergism or cooperation of both TF during the inflammatory response. However AP-1-dependent activation of genes such as those encoding MMPs is thought to contribute to clinical complications in several inflammatory disease state, example RA and atherosclerosis [201, 202].

2.3.3 NUCLEAR RECEPTORS IN THE REGULATION OF INFLAMMATORY RESPONSE

The NR superfamily has diverse roles in regulation of developmental reproductive homeostatic, metabolic, inflammatory and immune processes [203, 204]. NRs have highly conserved domain structure; the N-terminal region contains a ligand-independent activation function domain (AF1) and a zinc-finger DNA binding domain (DBD), the C-terminal region contains ligand-independent activation function domain (AF2) and ligand-binding domain (LBD), which binds receptor specific ligands [205, 206]. NRs can be divided into three subclasses. The first consists of the classical (prototypical) steroid hormone receptors, including progesterone receptor estrogen receptor and GR. The second subclass contains the so-called orphan receptors, the regulatory ligands of these receptors are undefined or do not seem to be required. The third subclass consists of ligand-dependent NRs such as thyroid hormone receptor, retinoic acid receptors (RXRs), liver X receptors (LXRs) and PPARs [207]. These NRs are activated by a diverse group of endogenous and exogenous small molecules. The members of this subclass form heterodimer with RXR and bind to DNA on promoter region of target genes. Heterodimers recognize sequence specific binding sites, response elements. Unliganded

heterodimers recruit corepressor protein and inhibit transcription of target genes, upon ligand-activation interact with coactivators and induce gene transcription [206, 208].

Several studies showed that NRs have major role in the negative regulation of inflammatory gene expression and immune response by several different mechanisms, including ligand-dependent inhibition of gene expression through sequence-specific binding to negative regulatory elements, positive regulation of anti-inflammatory gene expression and ligand–dependent transrepression. Mostly GR and PPARs are involved and extensively studied in the anti-inflammatory gene expression regulation [191, 209].

2.3.3.1 GR

GR is a member of NRs, which has an important role in immunosuppression and regulation of anti-inflammatory processes [210]. In the absence of its ligand GR forms homodimer and binds to heat shock protein (HSP)-90 and located in inactive state in the cytoplasm [211]. Corticosteroids can bind to GR, which cause the dissociation of HSP-90 from the homodimer and translocation of GR into the nucleus, where GR recruits HDAC2 and binding to the activated NF-κB suppresses inflammatory gene expression [212] (Figure 8).

Corticosteroids are highly effective in the treatment of asthma and other chronic inflammatory and immune diseases. However, there is a small population of patients with asthma fail to respond to corticosteroids and patients with COPD also show a poor clinical response. The mechanism of steroid resistance may be explained by an inhibitory effect of cigarette smoking and increased oxidative stress, which inhibits HDACs. The formation of peroxynitrite, which is generated from superoxid anions and nitric oxide, causes the nitration of HDACs [199, 213]. Nitration of protein alters protein function, reducing enzyme activity and makes proteins more susceptible to protein degradation via proteasome [214, 215]. Decreased HDAC activity was showed in cigarette smoke extract (CSE) induced macrophages, while high expression of IL-8 and IL-1β were found at the mRNA level [44, 216].

GR also can inhibit inflammation via induction of gene expression of anti-inflammatory genes such as mitogen-activated protein kinase phosphatase-1 (MKP-1) or secretory leukocyte protease inhibitor (SLP1) [212].

2.3.3.2 PPARs

PPAR family consists of three members: PPARα, PPARδ and PPARγ. They show different tissue distributions and have distinct and also overlapping biological functions [217-219]. PPARs form heterodimers with RXR and bind to sequence-specific PPAR response element (PPARE) in the promoter of their target genes. PPAR family modulate various cellular functions such as adipocyte differentiation, fatty-acid oxidation, glucose metabolism and they have important role in the inhibition of inflammatory gene expression [220]. PPARs can repress target genes of NF-κB, nuclear factor of activated T cells (NFAT), AP-1 and signal transducer and activators of transcription (STATs) [208, 221-223].

PPARα is expressed in the liver, brown fat and heart, involved in regulation of cellular energetics and also have anti-inflammatory role of which mechanism incompletely understood [224]. Using PPARα ligands a reduction in the symptoms of inflammation and disease were shown in different disease models, including allergic airway disease, RA and IBD [225].

PPARδ is ubiquitously expressed and involved in fatty-acid metabolism, mitochondrial respiration, thermogenesis and the programming of muscle fiber, but its role in the modulation of inflammation is poorly understood [226, 227]. It was shown that PPARδ ligand could upregulate arginase 1, which is a marker of alternative activation of macrophages [228].

2.3.3.2.1 PPARγ

Four variants of the PPARγ mRNA are known, the PPARγ1, PPARγ2, PPARγ3 and PPARγ4, which arise due to alternative splicing/promoter usage [229]. PPARγ can be activated by fatty acid derivatives, such as 15-deoxy-$\Delta^{12,14}$ -prostaglandin J_2 (15d-PGJ_2), which are natural ligands of PPARγ and by thiazolidinediones (TZDs), for example rosiglitazone [230, 231]. PPARγ is abundantly expressed in adipose tissue and in many other tissues and different types of cells including the lungs and AMs. It plays a central a role in adipogenesis, intracellular insulin signaling and regulation of cellular energy homeostasis and involved in alternative macrophage activation and DC biology [217,

220]. PPARγ also has an anti-inflammatory effect, inhibiting the expression of pro-inflammatory genes by interfering with NF-κB and AP-1 or inducing transcription of anti-inflammatory genes. Expression of IκB, the inhibitory protein of NF-κB is positively regulated by PPARγ (Figure 8) [221-223].

Several studies have shown that PPARγ ligand treatment can ameliorate inflammatory response in the lungs, joints, nervous system and gastrointestinal tract [232, 233]. Animal models of various airway diseases such as asthma, pulmonary fibrosis, acute lung injury and COPD have been examined the anti-inflammatory effects of PPARγ ligands [234]. Administration of rosiglitazone reduced neutrophilic inflammation in the lungs of mice exposed to intratracheal endotoxin, which is associated with reduced expression of CXC chemokines and GM-CSF [235-237]. PPARγ agonists block the profibrotic effect of TGF-β on fibroblast and reduce pulmonary fibrosis in animal models and also inhibit the TGF-β induced differentiation and collagen secretion of human lung fibroblast and myofibroblast [238]. These findings suggest that PPARγ agonists might reduce small airway fibrosis in COPD and recently a clinical trial examined the effect of rosiglitazone on lung function in comparison with low-dose inhaled corticosteroids in steroid-naïve smokers with asthma [239]. However cardiovascular side effects of TZDs have recently been reported and their usage in the treatment of diabetes is limited [240-242].

3 AIMS

Development of chronic inflammatory diseases is combination of environmental and genetic factors. Identification of associated genetic factors enables to predict the susceptibility to the disease and give potential targets to develop new effective therapies. Since COPD and IBD show increasing frequencies in Hungary in recent years, we decided to perform global gene expression and genetic association studies in Hungary [243].

The objectives of our studies

1. To expand and validate the list of genes associated with smoking-induced COPD in AMs.
2. To find and validate potential biomarkers in peripheral blood MOs that reflect COPD-specific gene expression patterns identified in AMs.
3. To correlate gene expression patterns with disease parameters.
4. To investigate the association of EPHX1, SERPINE2, TNF-α and SFTBP polymorphisms to COPD in a Hungarian population.
5. To assess possible association between SNPs of PPARγ (rs10801282 (Pro12Ala), rs3856806 (His447His) and rs1800571 (Pro113Gln)), a new candidate gene, and COPD outcome.
6. To examine the association of four polymorphisms of PPARγ (rs10865710 (C-681G), rs2067819, rs3892175 and Pro12Ala) with CD and UC in a cohort of Hungarian patients.

4 METHODS

4.1 Study population

4.1.1 INFORMED CONSENT

The Research Ethics Committee of University of Debrecen Medical and Health Science Center approved the clinical protocol and the study. Written informed consent was obtained before the subjects enter the study. The investigator explained the nature, purpose and risk of the study and provided the subject with a copy of the information sheet. The subjects were then be given time to consider the study's implication before deciding to participate.

4.1.1.1 Inclusion/exclusion criteria for COPD study

Before starting sample collection we defined the inclusion and exclusion criteria of diseased and healthy patients. All patients (350 COPD and 350 healthy control patients) were recruited from Eastern Hungary with Caucasian origin.

Inclusion criteria for COPD subjects were age 40 to 65 years old. Patients must have predicted value of FEV_1 <80% and FEV_1/FVC% < 70% (stage 2 and 3 according to GOLD criteria). Patients must show <15% reversibility of airway obstruction to a standard inhaled dose of salbutamol (Ventoline 2 puffs of 100μg) (www.goldcopd.com). Patients were excluded from the study who had unstable COPD, such as exacerbations in the 3 previous month (using physician's diagnosis) or reversibility > 15% of airway obstruction to a standard inhaled dose of salbutamol. Inclusion criteria for control patients were age between 40 and 65 years, must have normal spirometry, $FEV_1 \geq 80\%$ (predicted value) and FEV_1/FVC% ≥ 70%. All patients must be current or ex-smoker (minimum 15 pack years).

Exclusion criteria for all patients:

1. Plasma IgE level > 120U/ml,
2. Alpha1-antripsin deficiency,
3. Evidence of asthma,

4. Atopic disease
5. History of lung disorders such as childhood lung disease, cystic fibrosis, bronchiectasis, tuberculosis and occupational lung disease
6. History of respiratory infection in the past 3 months,
7. Other inflammatory diseases such as IBD (CD and UC), RA, osteoarthritis, psoriasis, eczema etc
8. History of autoimmune disease such as systemic connective tissue disease Sjögren's disease, Lupus (SLE), systemic sclerosis, etc.
9. History of other clinically significant disease such as on-stable congestive heart failure, recent myocardial infarction (in the previous 12 months) unstable angina, diabetes, renal disease, cancer
10. Positive plasma test for HIV, Hepatitis B or Hepatitis C.

Medication:

1. History of oral, inhaled intranasal or topical corticosteroid or immunosuppressive drug treatment in the previous 3 months
2. History chromoglycate treatment in the previous 8 weeks
3. History of leukotriene modulator treatment in the previous 8 weeks
4. History of antibiotic treatment in the previous month
5. History of theophyllin treatment in the previous 3 days (short-acting) or 1 week (long-acting)
6. History of anticholinergic treatment in the previous 8 hours (short acting) or 1 week (long acting)
7. History of beta2-agonist treatment in the previous 8 hours (short acting) or 12 hours (long acting)
8. Vaccination against Influenza or S. pneumonia in the last two weeks

4.1.1.2 Patient cohorts of IBD study

Patients were recruited from four locations by four centers (all of them are members of the Hungarian IBD Study Group). 675 well-characterized IBD patients (CD 572, men/women 264/292, age 36.4 ± 12.6 and UC 103, men/women 48/55, age 41.5 ± 15.5) and 486 healthy controls (blood donors) were examined.

The diagnosis was based on the Lennard-Jones criteria [244]. Age; age at onset; presence of EIM; arthritis: peripheral and axial; ocular manifestations: conjunctivitis, uveitis, and iridocyclitis; skin lesions: erythema nodosum, and pyoderma gangrenosum; and hepatic manifestations: primary sclerosing cholangitis (PSC), frequency of flare-ups (frequent flare-up >1/yr), therapeutic effectiveness (*e.g.* need for steroid and/or immunosuppressive therapy, steroid resistance as defined by the European Crohn's and Colitis Organisation Consensus [ECCO] Report [245], or short-term response to infliximab therapy), need for surgery (resections), the presence of familial IBD, and smoking habits were investigated by reviewing the medical charts by the physician and by completing a questionnaire. In CD, an additional parameter, perianal involvement, was also investigated. The disease phenotype (age at onset, duration, location, and behavior) was determined according to the Montreal Classification (non-inflammatory behavior: either stricturing or penetrating disease) [246]. Only patients with a confirmed diagnosis for more than 1 year were enrolled. In UC the disease extent was defined by the maximum extent during follow-up according to the Montreal Classification [247]. Only patients with a confirmed diagnosis more than 1 year were enrolled.

486 sex- and aged-matched healthy control blood donors (men/women 231/260, age 40 ± 12.6) were recruited. Control patients had no history of cancer, IBD and other chronic inflammatory or autoimmune diseases (e.g. RA, psoriasis, SLE).

4.2 BAL procedure

After pre-medication with atropin (0.5 mg i.m.) local anesthesia of nostrils mouth and throat was achieved with lidocaine spray. Fiber-optic bronchoscope (Olympus) passed through the nose, pharynx, large airways and the tip of a total 300 ml 0.9% physiological saline solution sterile warm (37 Celsius degree), in 50 ml aliquots. For intermediate aspiration using same 50 ml syringe. Each aliquot transferred into plastic tubes and placed immediately on melting ice.

4.3 Alveolar macrophage separation

BALF samples volumes were 100-150 ml and samples were filtered with sterile gauze to eliminate mucus and centrifuged at 1500 RPM for 10 minutes at 4-Celsius degree to collect the cells. After resuspending in 1 ml PBS alveolar macrophages were separated by Percoll (Amersham Biosciences) gradient centrifugation. Percoll was pre-centrifuged at 15000 RPM for 20 minutes at 4-Celsius degree and then cell suspension was layered on it. Then we centrifuged at 3000 RPM for 30 minutes at 4-Celsius degree. During the centrifugation AM cells formed a cell ring in Percoll phase. We collected alveolar macrophages from there, washed in 10 ml PBS. Total cell number was determined by counting in hemocytometer. We carried out differential cell count on hematoxilin-eosin stained cytospin slides before and after the gradient separation. Cell viability was checked by Trypan blue exclusion. After the separation >95% AM purity was reached (AM 95-98% 96.6 ± 1.3%, lymphocytes 0.5-4% 1.7 ± 1%, neutrophils 1-2.4% 0.8 ± 0.6%) [248].

4.4 Peripheral blood monocyte separation

50 ml heparin treated venous blood was collected from healthy and diseased patients. Blood samples centrifuged at 2500 RPM for 20 minutes, then the supernatant (plasma) was removed and all of the cells were resuspended in 20 ml of physiological saline and layered on Ficoll, then centrifuged at 1700 RPM for 15 minutes to separate peripheral blood mononuclear cells (PBMC). Layer of PBMCs were collected and washed in 20 ml of physiological saline, centrifugation step was repeated to collect the cells. Cell number was determined and based on it, accurate volume of buffer and CD14 magnetic beads were added. After 15 minutes incubation at 4-Celsius degree monocytes were separated in magnetic field (>98% MO, 98-99% MO) (VarioMACS, Miltenyi Biotec.).

4.5 RNA processing

Total RNA from alveolar macrophages and peripheral blood monocytes were purified using RNeasy Mini Kit (Qiagen). RNA quality was determined Agilent

Bioanalyser 2100 (Agilent Technologies), RNA sample with >9.0 RIN number and 28S/18S ratio=1.6-2.0 was used for microarray analysis. RNA concentration was measured on NanoDrop ND-1000 instrument (UV spectrophotometer). Affymetrix HG-U133A arrays (Affymetrix) were used to perform gene expression analysis. In BALF collection we used every sample separately, but we made a control and COPD RNA mix from 5-5 patients in the case of MOs.

Complementary DNA (cDNA) was generated from 5 µg total RNA, using SuperScript choice system (Invitrogen) Double stranded cDNA was purified by phenol chloroform extraction and precipitation. This material was used for in vitro transcription (IVT). We used BioArray™ HighYield™ RNA Transcript Labeling Kit (T7) (ENZO Life Sciences) to synthesize biotine labeled cRNA. We used the RNeasy Mini kit to purify our cRNA samples. We fragmented 20 µg labeled cRNA by 5x Fragmentation Buffer (200 mM Tris-acetate, pH 8.1, 500 mM KOAc, 150 mM MgOAc). During the process we checked the quality of cDNA, cRNA and fragmented cRNA on agarose gel. We sent our fragmented samples to hybridize and scan to EMBL Genomics Core Facility (Heidelberg).

4.6 Microarray data analysis

AM and MO microarrays were analyzed in separate experiments using GeneSpring 7.3 software (Agilent Technologies). Affymetrix data files were imported directly into Genespring using GCRMA algorithm and median normalization was performed. First we determined the list of genes that showed a gene expression fold ratio of at least 1.5 up or down, compared to median in each sample. Then, based on this list we performed separate statistical analyses (Mann-Whitney non-parametric test) for each gene to identify differentially expressed genes over the probability level $P < 0.05$. We are aware of the cumulative effect of Type I error in such multiple situations, therefore these tests served the purpose of selecting genes for further study rather than formal tests of significance.

4.7 TaqMan Real-Time Quantitative Polymerase Chain Reaction (RT-QPCR)

In order to validate the microarray results, we used TaqMan Low Density Arrays (TLDA) and individual gene expression assays (Applied Biosystems). To confirm AM and MO common genes we used Roche Universal Probe Library system (UPL probes). TLDA contained 96 genes. 4 of them were housekeeping genes: beta-actin (ACTNB), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), peptidyl-prolyl isomerase A (PPIA), ribosomal phosphoprotein large P0 (RPLP0). 69 of the 92 genes were chosen from our microarray analysis. Probe sets that did not show an at least 1.7 fold change when the COPD group was compared to the control group were excluded from the assay. Expressed sequence tags, hypothetical protein coding probes were removed and only one probe set per gene was retained and genes with low expression levels (raw values < 50) were filtered out. Finally 69 genes were selected to which we have added another 23 candidate genes, which were known from previous COPD microarray studies, or expressed in macrophages.

First strand cDNA was generated from 5 ug total RNA using cDNA Archive Kit (Applied Biosystems). For RT-QPCR reaction 200 ng cDNA/sample and 2X TaqMan PCR mix (Applied Biosystems) was used. TLDAs were run in ABI Prism HT 7900 instrument. Two samples could be loaded on this TLDA format.

Independent RT-QPCR measurements were carried out and analyzed using the ΔΔ Ct method. Housekeeping genes were tested by geNorm software (http://www.wzw.tum.de/gene-quantification). According to our data, GAPDH is the least affected housekeeping gene in this system, therefore only GAPDH was used for normalize RT-QPCR data. Normalized results were statistically analyzed in GraphPad Prism 5.0 software (http://www.graphpad.com/), using non-parametric Mann-Whitney U-test.

4.8 Canonical variates analysis (CVA)

Separation between predefined groups of objects is best revealed by CVA, which avoids the problem of accumulating Type I errors in simultaneous individual univariate

tests. It was used to determine whether the groups of healthy and COPD patients are separable in the multidimensional space spanned by the genetic variables, and if so, which gene subsets have the best discriminatory power. The results of CVA are the so-called canonical scores obtained from the canonical functions derived through eigenanalysis, which serve as coordinates of observations in the canonical space. Due to the fact that the maximum number of canonical axes is one less than the number of groups, in the present study CVA did not allow graphical display, and separation of diseased and non-diseased patients is expressed merely by a list of scores for observations on a single canonical axis. If the observations are taken at random, variances are homogeneous and the variables satisfy multivariate normality, then statistical procedures are available to test the significance of group separation (χ^2 test). Wilks λ is another useful statistic to measure goodness of separation: the closer its value is to 0, the better the separation of groups. Nevertheless, if these criteria are not met, examination of the two groups whether they overlap on the canonical axis or not, provides equally meaningful information. A partial limitation of CVA is that the number of variables (genes) cannot exceed the number of observations (patients).

Computations were performed by the SYN-TAX 2000 package (Podani, J, 2001, SYN-TAX 2000. Computer programs for data analysis in ecology and systematics. User's Manual. Scientia, Budapest) [249, 250].

4.9 DNA isolation

Venous blood samples were collected in tubes containing EDTA, and stored at minus 20 Celsius degrees until DNA isolation. Genomic DNA was extracted from 0.5 ml whole blood using Roche MagNa Pure LC instrument and MagNa Pure LC DNA Isolation Kit (Roche Life Sciences) according to the manufacturer's protocol. DNA concentration was checked by UV photometry using NanoDrop ND1000 instrument (NanoDrop Technologies).

4.10 SNP genotyping

SNPs were genotyped using TaqMan SNP genotyping assays (Applied Biosystems). Following PCR amplification, the end-point fluorescence was read with the ABI 7900 HT Prism instrument and genotypes were assigned using the allelic discrimination option of SDS 2.1 software. The average genotyping success rate of at least 95% was attained for each SNP.

4.11 Immunohistochemistry

Tissues for morphology and immunostainings were obtained from the files of Pathology Department of University of Debrecen. They were freshly fixed in 10% neutral formalin and embedded in paraffin followed by hematoxyline and eosine (HE), elastic van Gieson, Masson-trichrome stainings using standard methods. Immunohistochemistry (IHC) for AMs was carried out using PPARγ and DCSign (Santa Cruz) monoclonal antibodies (mab) by means of immunoperoxidase staining as described earlier. Double immunofluorescence was made as described earlier using CSAII detection kit with FITC-labeled tyramine followed by an immunofluorescent staining for DCSign with streptavidin-texas red fluorochrome [251]. Nuclear counterstaining was made with DAPI (blue fluorescence).

4.12 Statistical analysis of SNP study

Differences between cases and controls concerning demographic and main clinical data were analyzed using Mann-Whitney *U*-test and Pearson χ^2 test. Genotype data for each SNP were tested for departures from Hardy-Weinberg equilibrium (HWE) separately in case and control populations using a goodness-of-fit χ^2-test or the exact test to estimate P values [252]. HWE calculations were done by using the HWE tool, it is available on http://ihg.gsf.de/cgi-bin/hw/hwal.pl. The significance of differences in genotype and allele frequencies between patients and controls were tested by using either χ^2 analyses or Fisher's exact test where appropriate. To assess the degree of association between each of the SNPs and disease conditions odds ratios with 95% confidence intervals (OR, 95% CI) were calculated using logistic regression analysis; the model was

adjusted for SNPs, pack-year, age and gender in the case of COPD. All single locus association tests were performed using the STATA 9.0 statistical package (except where otherwise stated).

Haplotype frequencies were estimated for control and patient groups separately with the Full-Precise-Iteration algorithm implemented in the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php). The extent of linkage disequilibrium (LD) between pairs of biallelic markers was determined using both the standardized disequilibrium and correlation coefficients (given as Lewontin's D' and $r^{2,}$ respectively) and association between haplotypes and disease conditions was assessed by the χ^2-test or the exact test as implemented in the program SHEesis [253, 254]. Correction for multiple testing was not used in the analysis of the association genotype and allele frequencies because: (i) the EPHX1 polymorphisms were known to be functional and (ii) the gene is considered a susceptibility gene for COPD; and (iii) in case of the PPARγ polymorphisms the studied individual alleles were not independent. *A posteriori* estimates of study power were assessed by means of Quanto software (http://hydra.usc.edu.gxe).

Power of COPD study

We have estimated the power of our study with the following parameters: sample size of 272 cases; control/case ratio of 1.1; minor allele frequencies (MAF) are in the range from 0.12 to 0.3; log-additive model; disease prevalence of COPD 5%. Assuming these parameters our study had ~50% power to detect a genotype relative risk (GRR) of 1.4 for MAF = 0.12, or ~79% power to detect a GRR of 1.6 for the same MAF; while it had ~76% power to detect a GRR of 1.4, and ~96% power to detect a GRR of 1.6 for MAF = 0.3 at an α=0.05 significance level.

Power of IBD study

The power of our study was estimated with the following parameters: sample size of 572 cases with control/case ratio of 0.86 and disease prevalence 0.11% for CD; 103 cases with control /case ratio of 4.77 and disease prevalence 0.21% for UC. Minor allele frequencies (MAF) were in the range from 0.10 to 0.28 for all SNPs. Assuming these parameters our study had ~98% power to detect a genotype relative risk (GRR) of 0.3 for minor allele frequency = 0.11 in case of the CD cases at an α=0.05 significance level. In

case of the UC cohort, our study had ~54% power to detect a GRR of 1.6 for minor allele frequency = 0.10 at an α=0.05 significance level.

5 RESULTS

5.1 Gene expression analysis of COPD

5.1.1 EXPLORATORY MICROARRAY ANALYSES OF AMS OF BALF SAMPLES

BALF samples were collected and AMs were separated from 5 controls and 5 COPD patients all of whom were active smokers. Patients with COPD were stage 2-3 patients according to GOLD. The control and COPD groups were homogenous regarding age, smoking habits, but differed in lung function parameters and CRP levels (Table 1).

Parameter		AM exploratory cohort		AM validation cohort		MO validation cohort	
		Control	COPD	Control	COPD	Control	COPD
n		5	5	20	26	16	22
Sex (M/F)		5	5	19/1	24/2	13/3	21/1
Ancestry		Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Age	mean ± SD	44.8± 7.6	52.8 ±6.6	53.7 ± 13.7	57.8 ± 9.4	53.4± 7.6	57.5 ±9.2
	range	40-58	48-63	42-64	43-65	47-65	44-65
Smoking Status							
Current		5	5	17	22	14	19
Former				3	4	2	3
Pack-years (packs)	mean ± SD	31.26 ± 25.9	40.2 ± 17.5	29.1 ± 14.3	35.9 ± 18.1	32.1 ± 20.9	35.7 ± 17.2
	range	15-58	29-70	15-60	16-76	20-78	15-75
Duration of smoking (years)	mean ± SD	19.4 ± 12.26	30.2 ± 10	30.9 ± 14.1	34.3 ± 11.8	27.4 ± 13.2	36.1 ± 10.5
	range	9-39	15-42	9-43	12-47	23-49	25-45
FVC% (predicted value)*	mean ± SD	90.1 ± 6	74.5 ± 4.1	92.6 ± 8.9	71.5 ± 7.9	96 ± 14.1	65.1 ± 12.4
	range	82-96.2	65.8-79	81-116	42-79	81-103	36-73
FEV1% (predicted value)*	mean ± SD	88.8 ± 8.1	55.8 ± 14.8	93.2 ± 8.6	63.1 ± 14.3	96.8 ± 12.3	70.2 ± 7
	range	80.8-100.3	36.6-76	83-112	32.5-78	83-109	31-78
BMI (kg/m^2)	mean ± SD	26.8 ± 3.6	23.5 ± 2.7	26.2 ± 2.6	25.2 ± 3.7	27.6 ± 3.8	26.2 ± 5.4
	range	24.1-32.28	20.07-26.8	20.1-35	18.6-31.1	23.8-35.1	18.7-36.75
CRP (mg/L)*	mean ± SD	2.3 ± 1.9	15.7 ± 11.3	2.6 ± 1.4	6.3 ± 4.3	2.6 ± 1.7	6.4 ± 4.7
	range	0.5-3.6	2.1-18	0.33-5.1	1.16-17	0.5-6.2	1.19-4.79

Table 1. Parameters and characteristics of the COPD patients and controls

Data are presented as means ± SD and ranges. * $p < 0.05$, COPD patients vs. controls (Mann-Whitney U test).

AMs of 5-5 control and COPD patients were subjected to global gene expression analysis using microarrays (Figure 9A). We identified a list of 671 probe sets representing 389 annotated genes, which showed significant correlation with the disease. Hierarchical cluster analysis was performed and these 671 probe sets separated completely healthy controls from COPD patients (Figure 9B).

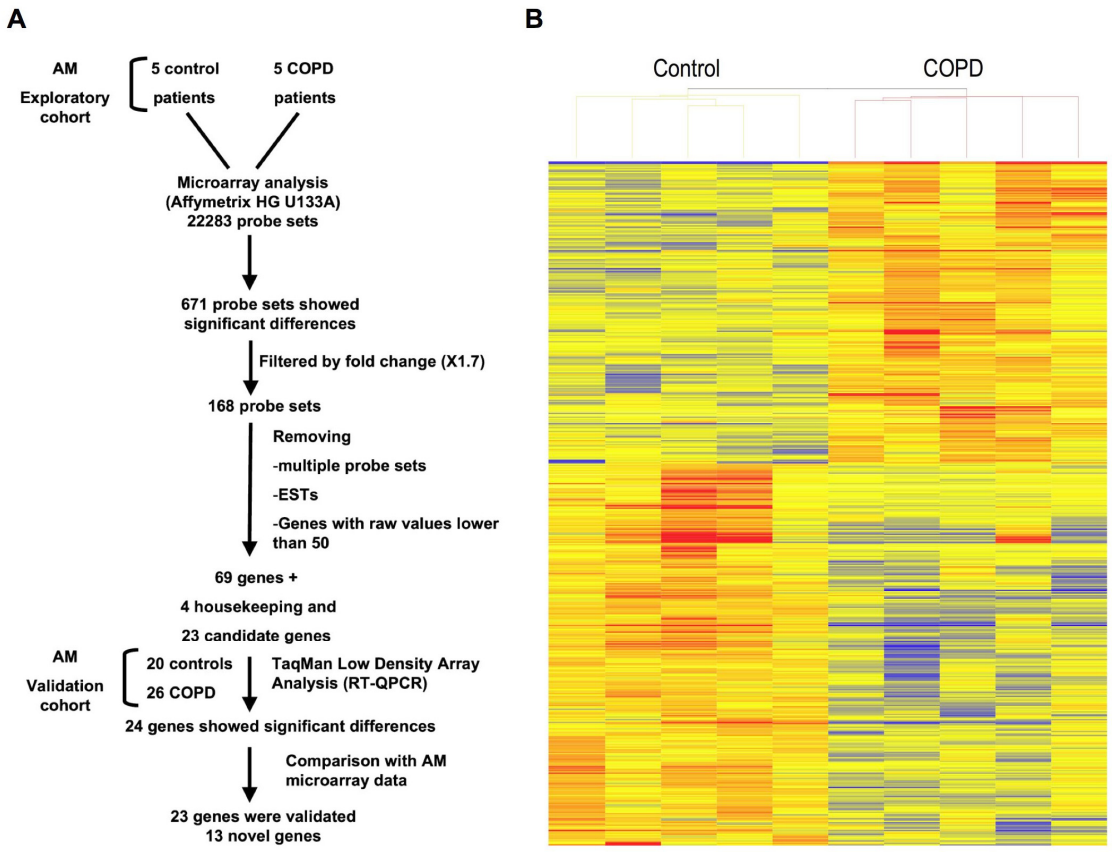


Figure 9. Experimental design of gene expression analysis

A. AM were collected from healthy and diseased patients using fiber-optic bronchoscopy. After RNA isolation samples were hybridized to Affymetrix HG U133A GeneChips Microarray data were analysed using GeneSpring 7.3 software. 22283 probe stets were tested by filter on expression level option, 671 probe sets showed significant differences, of which 168 probe sets showed at least 1.7 fold change difference between control and COPD. Multiple probe sets, expressed sequence tags and genes with raw values less than 50 were removed resulting in a list of 69 genes. 96 genes including these 69 genes were analyzed on TLDA on which 24 of them showed significant differences and 23 were validated.

B. 671 probe sets (representing 389 annotated genes) showed significant differences between healthy control and COPD patients in the AM microarray analysis. Unsupervised hierarchical cluster analysis was

performed on this list using Pearson correlation. These probe sets completely separated the healthy and diseased group.

Comparison of the transcripts expressed differently in disease versus control samples revealed some previously identified COPD associated genes, including CYP1B1, a cytochrome P450 enzyme known to be associated with smoking, and induced by cigarette smoke. Phospholipase A2 (PLA2G7) has a role in immune response, and is also upregulated in COPD. Chemokine ligand 2 (CCL2), osteopontin (SPP1), IL-8 and IL-1β genes are involved in immune response, chemotaxis, and cell migration [150, 151, 255]. These findings validated our data set and suggest that our novel findings can complement already existing markers and very likely to link additional genes and pathways to COPD.

5.1.1.1 Functional classification of COPD-specific genes

Next we sought to carry out a global functional classification of the identified signature gene set. We carried out gene ontology (GO) analysis using find significant functional categories. 389 genes, the results of the ANOVA analysis, were tested by hypergeometric test with Benjamini & Hochberg False Discovery Rate correction. We found that the identified genes have fallen into several of those pathways that are believed to be related to COPD pathogenesis and inflammatory processes (Figure 10). Importantly, we have identified some categories with high significance level representing response mechanisms of external and internal stimuli.

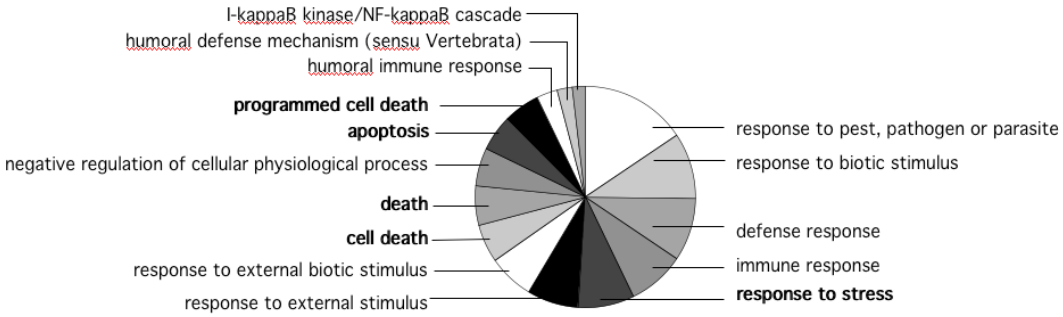


Figure 10. Functional categories of differentially expressed genes in COPD in AM experiment

Functional categories of the 389 genes, which showed different expression patterns between COPD and healthy patients in AM based on microarray analysis. Cytoscape 2.0 with Bingo plugin was

used to obtain overrepresented GO Biological Process categories. The 15 most significant categories are showed. Highlighted categories are overlapping with functional analysis of the peripheral blood MO expression pattern.

These categories contain several, in some cases overlapping, gene sets, which are coding for inflammatory and chemotactic cytokines e.g. IL-8, CCL2, TNF-α and other inflammatory genes (PLA2G7). Some of these genes are also involved in immune response and macrophage activation. We also identified genes with roles in apoptotic and cell death related pathways (PHLDA2) or cell cycle regulation (CDKN1A, GADD45A). Therefore the altered gene expression patterns indicate a change in AMs in response to stimuli and alterations in cell death/apoptosis processes.

5.1.2 CONFIRMATION OF CANDIDATE GENES BY RT-QPCR

In order to confirm and validate the microarray results, the gene expression levels of 96 selected genes were measured in the BAL samples of a new cohort of 46 patients including 26 COPD and 20 healthy controls. All individuals were active or ex-smokers in the validation set (Table 1).

TLDA, an RT-QPCR platform, was used for determining COPD-associated genes in AMs separated from BALF samples. Our custom TLDAs contained 96 genes including 4 housekeeping genes, 23 candidate genes obtained from the literature and 69 genes chosen from our exploratory microarray experiment. The list of 69 genes was created by filtering the 671 probe sets that correlated significantly with the disease in the exploratory microarray experiment by ±1.7 fold change compared to the median of all samples and removing multiple probe sets, expressed sequence tags and genes with raw values lower than 50 (Figure 9A).

Twenty-four genes showed significantly different expression patterns between control and COPD patients. Twenty-three genes changed in the same direction both on the microarrays and in the RT-QPCR measurements**.** Five genes previously reported in other COPD microarray studies (IL-8, CDKN1A, FOS, PLA2G7, and SPP1) were confirmed in this experiment (Figure 11) [150, 151, 156].

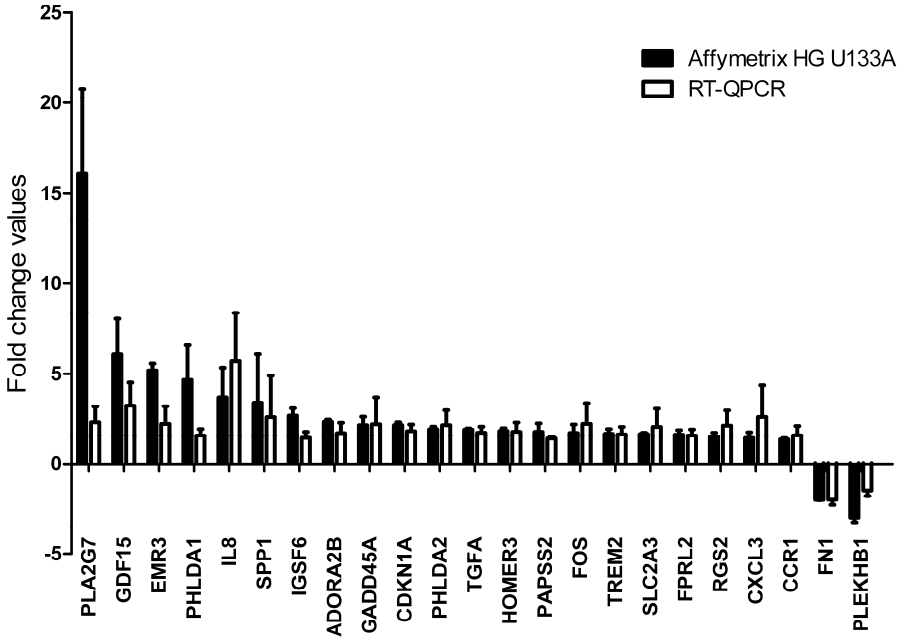


Figure 11. Comparison of AM microarray and RT-QPCR validation data

Fold differences as determined by microarray (HG U133A) and RT-QPCR in AMs (empty bars represent RT-QPCR, filled bars represent microarray data). These 23 genes showed statistically different expression levels between COPD and control patients (Mann-Whitney U test) and thus were validated.

Genes with already established roles in inflammatory processes of the lung were found, including ADORA2B, FN1 and TGF-α [256, 257]. Thirteen novel genes were found to be related to COPD inflammatory processes. These belong to the following functional categories: immune response (CCR1, TREM2), apoptosis (GADD45A, PHLDA1, PHLDA2) and signal transduction (EMR3, GDF15, FPRL2, HOMER3), suggesting that these functions are affected by the disease, while IGSF6, PAPSS2, PLEKHB1 and SLC2A3 were unclassified (Figure 12 A-D).

We performed an independent statistical approach, CVA using these 23 validated genes in order to test whether this gene set can indeed separate COPD patients from controls. Canonical correlation for this gene set was 0.8 (χ^2 =34.25, d.f. =20, significant at p=0.05, Wilks λ =0.365), which suggests that such a set can differentiate between healthy and COPD patients (Figure 12 E).

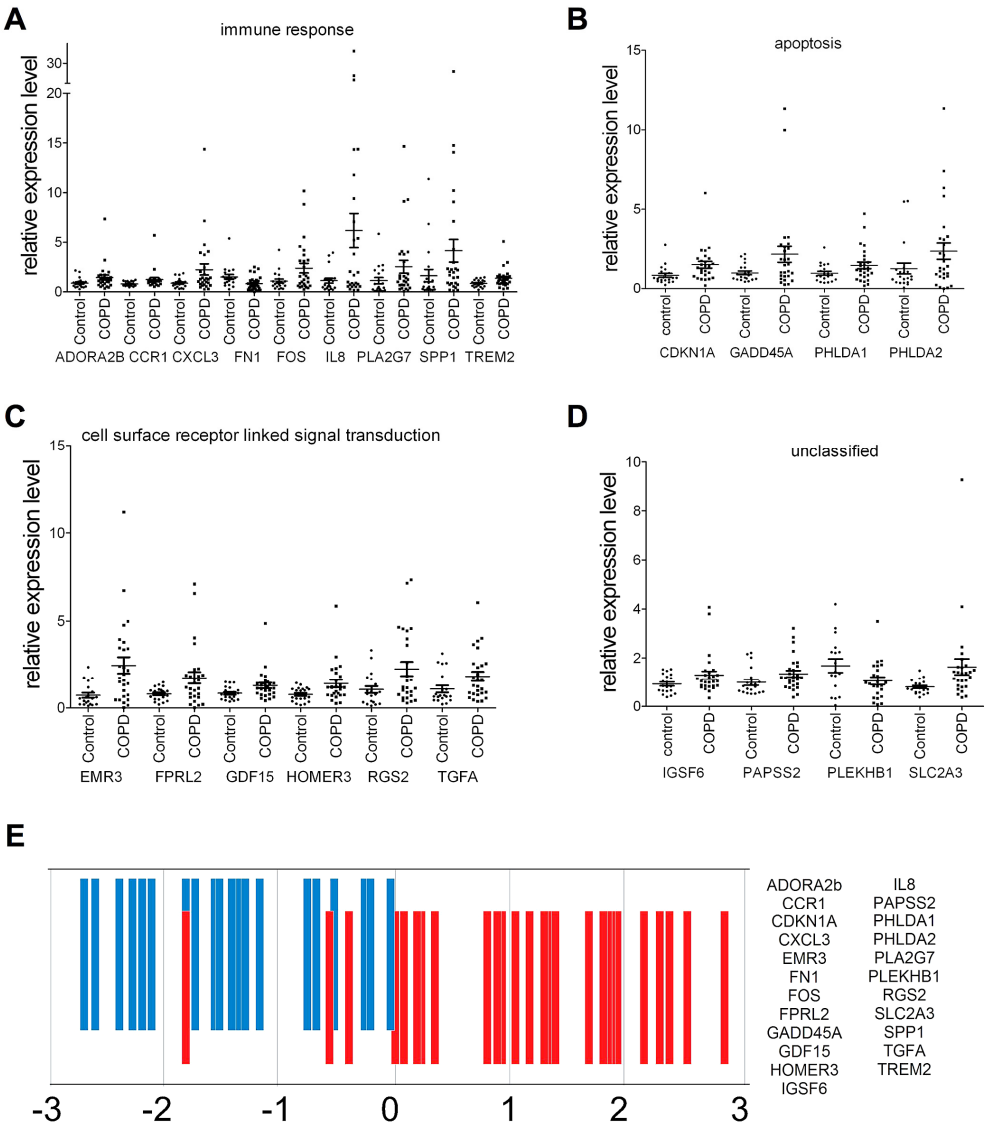


Figure 12. Individual values of RT-QPCR validated COPD-related genes and CVA

A-D. Validation of differential gene expression data by RT-QPCR. The individual values of RT-QPCR of the indicated genes from alveolar macrophages are plotted. Relative expression levels are shown normalized to GAPDH. All of the 23 genes showed statistically significant differences in expression levels between control and COPD patients (Mann-Whitney U test).

E. Canonical Variates Analysis of the validated 23 genes that showed significant differences between COPD and control patients in AM samples, separated COPD and healthy patients in the AM validation cohort. Red bars represent COPD, blue bars represent healthy patients. Numbers on axis X are spherized canonical scores of objects on the single canonical variate.

5.1.3 IDENTIFYING COPD-SPECIFIC GENE EXPRESSION SIGNATURES IN MOS

Next we decided to turn our attention to peripheral monocytes and explore their expression changes between COPD and control samples. MOs were separated from peripheral blood samples that were collected simultaneously and pooled separately from 5 controls and also from 5 COPD patients following RNA isolation. Microarray analysis was performed on this new sample set and results revealed a list of 555 probe sets with 356 annotated genes that showed at least 1.5 fold change differences between COPD and control patients in peripheral blood MOs. We compared that list to the AM microarray result. Comparison resulted in a list of 54 probe sets representing 35 annotated genes (Figure 13).

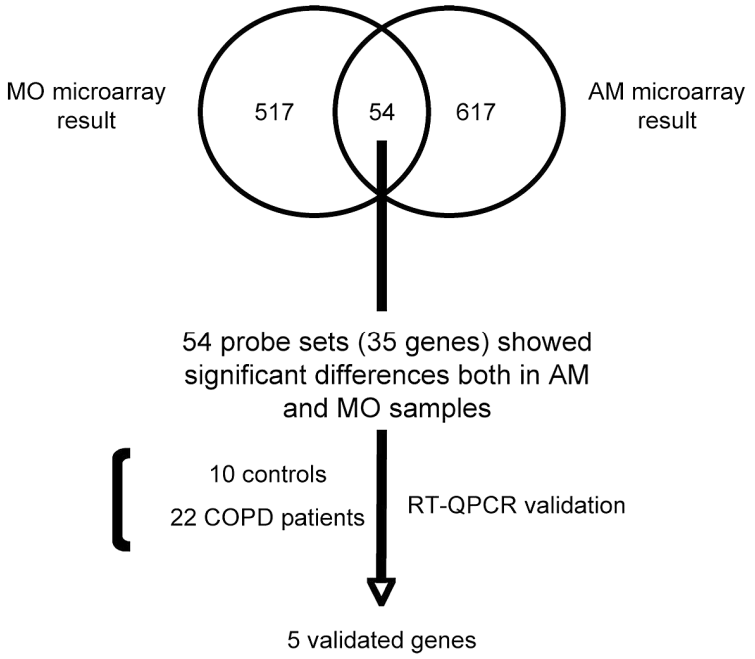


Figure 13. Experimental design of the comparison of AM and MO microarray data

Venn diagram of gene expression patterns of commonly expressed probe sets between AMs and MOs and probe sets expressed in a COPD specific manner in the two cell types, using HG U133A microarray data. 54 probe sets (representing 35 annotated genes) overlapped between AM and MO of which 5 genes were validated by using RT-QPCR.

GO analysis was also performed and interestingly the identified 356 genes have fallen into the response to stress and stimuli and cell death categories, as it was the case

with AMs samples. Remarkably, the majority of the identified categories overlapped with those found in disease effected AMs (Figure 14).

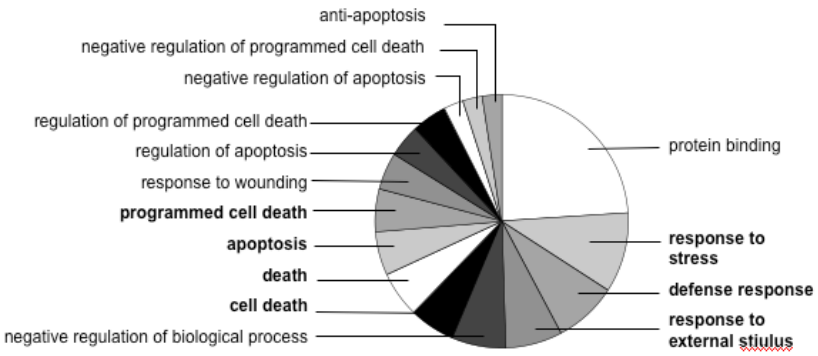


Figure 14. Functional categories of differentially expressed genes in COPD in MO experiment

Functional categories of the 356 genes that showed different expression patterns between COPD and healthy patients in peripheral blood MO based on microarray analysis. Cytoscape 2.0 with Bingo plugin was used to obtain overrepresented GO Biological Process categories. The 15 most significant categories are shown. Highlighted categories were overlapping with functional analysis of the AM expression pattern.

The overlapping categories are marked both on Figure 10 and Figure 14. The stress and response to external stimulus categories contained overlapping genes such as IL-8. The apoptotic and cell death related pathways also showed similarity between the two cell types and common genes were present e.g. the PHLDA2 gene. In addition we also found alveolar macrophage specific and peripheral monocyte specific functions, based on the COPD specific gene expression pattern. As expected, AMs are more affected in responses to internal stimuli and immune processes. Examining MOs we found that the protein-binding gene category contained most of the identified genes. Genes in this very broad category overlapped with other categories such as stress response or apoptosis related pathways. Although most of the apoptosis and cell death related categories were overlapping with AM, certain anti-apoptotic pathways were present only in MOs.

We validated commonly expressed genes in AMs and peripheral blood MOs by measuring the expression levels of these 35 genes using RT-QPCR. A new cohort of 22

COPD and 16 control patients was recruited/assembled and their MO RNA samples were examined for this validation, 13 COPD patients and 7 controls were newly recruited individuals (Table 1).

RT-QPCR analyses validated 5 of the 35 genes that showed significant differences between COPD and control patients. ADAM10, GK, IFRD1, PHLDA1 and SEC14L1 showed higher expression levels in diseased patients (Figure 15).

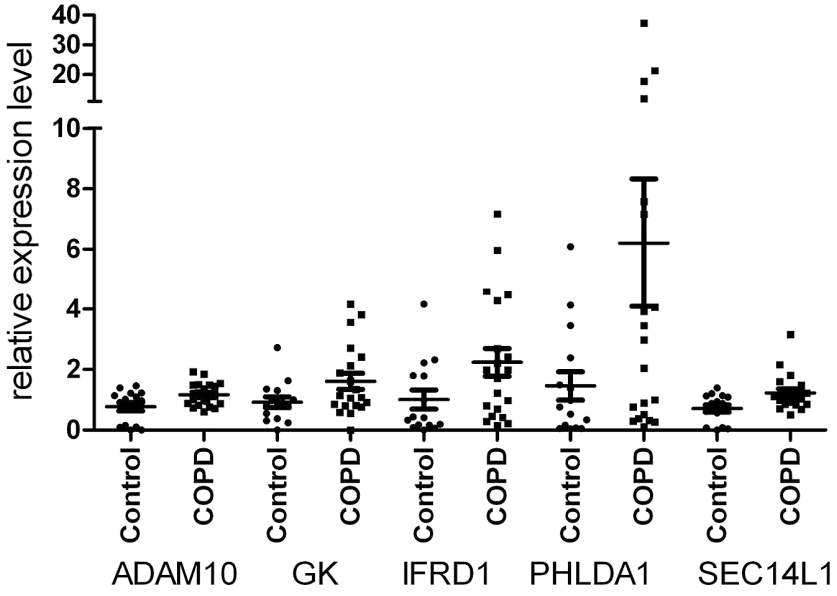


Figure 15. Validation of MO results by RT-QPCR

The individual values of RT-QPCR of the indicated genes from peripheral monocytes of patients of the cross validation set are plotted. Relative expression levels are shown normalized to GAPDH. All of the 5 genes showed statistically significant differential expression between control and COPD patients (Mann-Whitney U test).

CVA showed that the combination of these 5 genes separates fairly well the COPD and control patients (canonical correlation was 0.62, χ^2 =14.75, d.f. =5, significant at p=0.05, Wilks λ =0.617).

Remarkably, these experiments confirmed our hypothesis that peripheral monocytes also carry a COPD related gene expression pattern and moreover there is some overlap between AM and MO COPD signatures (Figure 16).

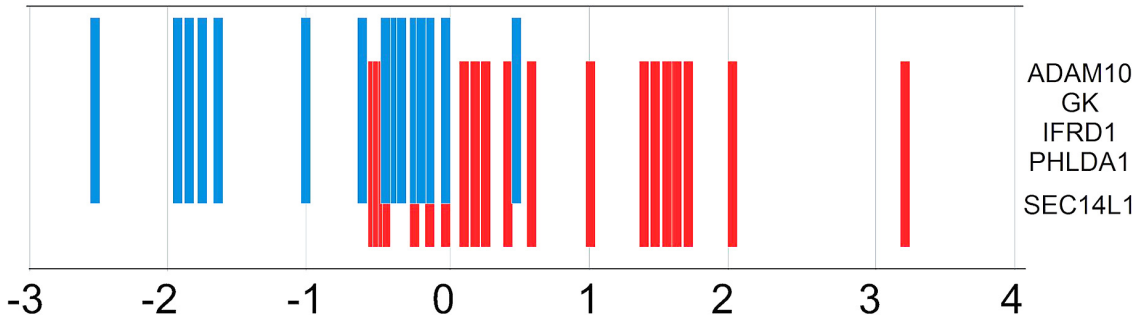


Figure 16. CVA of MO results

CVA of these 5 genes separated the COPD and control patients in the MO validation cohort. Red bars represent COPD, blue bars represent healthy patients. Numbers on axis X are spherized canonical scores of objects on the single canonical variate.

5.1.4 CORRELATION BETWEEN LUNG FUNCTION AND GENE EXPRESSION DATA

A key issue in identifying biomarkers in general and disease specific expression signatures in particular is the ability to correlate these with clinical parameters relevant in the disease. In the case of COPD, the key clinical feature is lung function decrease measured by lung function tests. Therefore, as the next step of our studies the possible correlation between FEV1% values and expression data of the RT-QPCR validated genes was examined. We calculated Pearson correlation between lung function parameter FEV1% and all the 23 genes and found that higher expression of CCR1, GDF15 and SLC2A3 from the AM validation set obtained from BAL samples correlated with lower FEV1% values. However, higher expression of FN1 correlated with increased values of FEV1% (Figure 17A).

ADAM10, GK, IFRD1 and SEC14L1 in the peripheral MO validation set showed correlation with lower FEV1% values, but PHLDA1 did not show correlation (Figure 17B). These data showed that gene expression changes can be correlated with lung function changes both in AMs and probably most intriguingly in peripheral blood MOs.

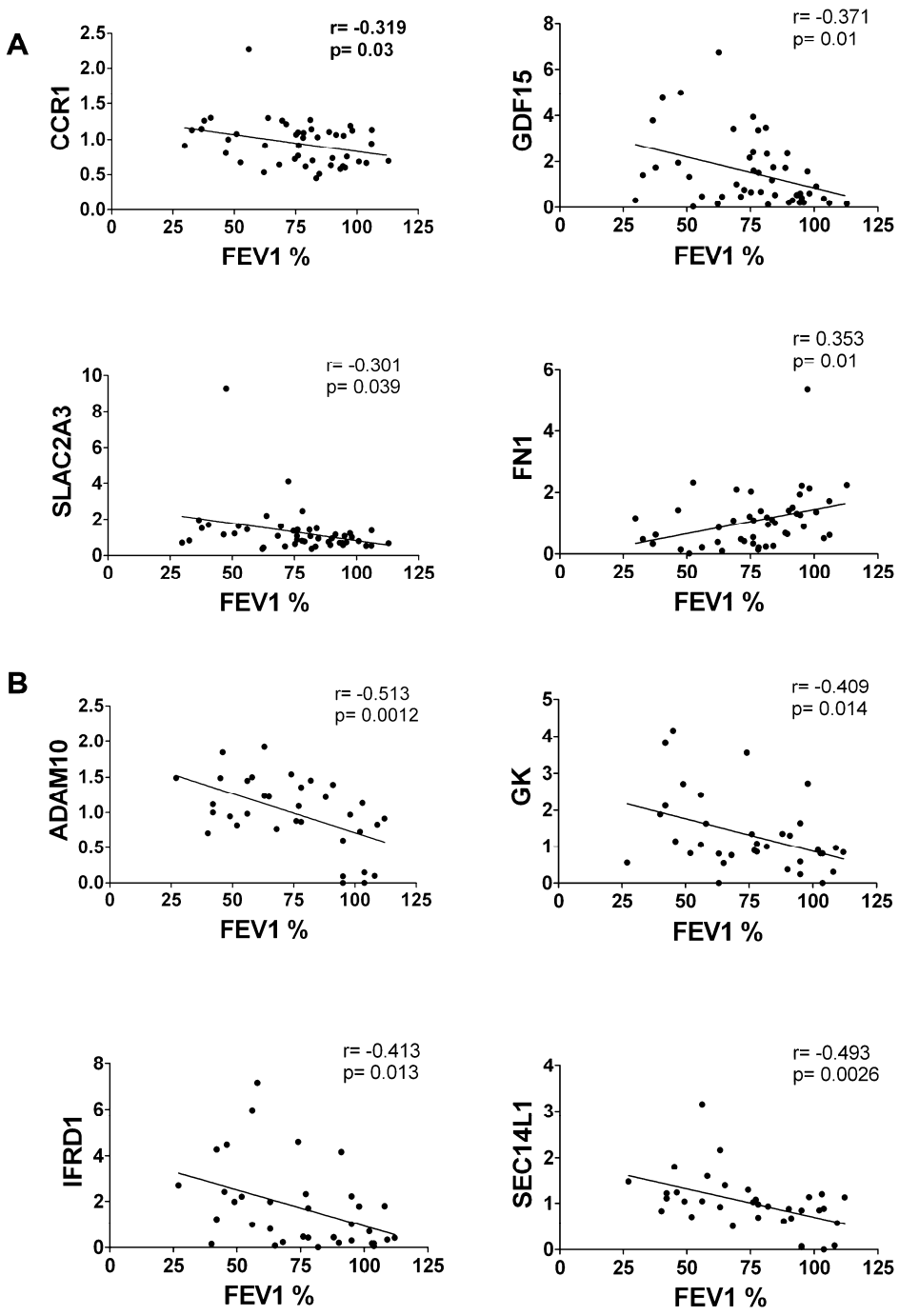


Figure 17. Gene expression signatures correlate with FEV1% (predicted)

COPD-specific gene expression signatures of AMs (notably CCR1, FN1, GDF15 and SLC2A3 on part A) and MOs (such as ADAM10, GK, IFRD1 and SEC14L1 on part B) correlate with FEV1% (predicted). Pearson correlation was used; statistical significance of correlations was tested at $p < 0.05$.

5.2 SNP analyses in chronic inflammatory diseases

5.2.1 COPD SNP STUDY

We genotyped 573 subjects, including 272 patients with COPD and 301 healthy controls. The proportion of males was higher among cases (69.85% to 54.48%) but there is no significant difference in the mean age of controls and cases. COPD patients had been exposed to more tobacco smoke as evidenced by the difference in pack-years but the difference is not significant, cases had a much larger reduction in lung function, typical for a clinical COPD population (Table 2).

Table 2. Clinical features of the COPD SNP study population

Parameter	Cases (N=272)	Controls (N=301)	P value
Male (%)	190 (69.85)	164 (54.48)	0.781
Age (±SD)	63.87 (±8.96)	64.29 (±9.07)	0.577
Pack-Years (±SD)	38.75 (±19.91)	34.76 (±14.71)	0.120
FEV1% predicted (±SD)*	47.17 (±13.69)	99.28 (±9.76)	<0.0001
FEV1/FVC% predicted (±SD) *	57.57 (±10.12)	87.10 (±35.24)	<0.001

Data presented as mean ±SD. FEV1: forced expiratory volume in one second, FVC: forced vital capacity, *Mann-Whitney U-test was used.

Table 3. Characteristics of examined SNPs

Gene Name	Gene Symbol	NCBI SNP reference number	SNP Type	Alleles and characteristics	ABI TaqMan genotyping assay ID
Serpin peptidase inhibitor, clade E	SERPINE2	rs6747096	Exon 3 A/G	Synonymous polymorphism	C___7614669_10
Microsomal epoxihidrolase 1	EPHX1	rs1051740	Exon 3 Tyr113His; C/T His; "slow" allele	Non-synonymous polymorphism	C_____14938_30
		rs2234922	Exon 4 His139Arg; A/G Arg; "fast" allele	Non-synonymous polymorphism	C__11638783_30
Tumor necrosis factor α	TNF-α	rs1800629	-308 G>A	promoter variant	C___7514879_10
Surfactin protein B	SFTPB	rs1130866	Thr138Ile; C/T	Non-synonymous polymorphism	C__11938981_20
Peroxisome proliferator-activated receptor γ	PPARγ	rs1801282	Exon B, Pro12Ala; C/G	Non-synonymous polymorphism	C__26856791_20
		rs1800571	Exon 3, Pro115Gln	Non-synonymous polymorphism	C___8756581_20
		rs3856806	Exon 6, His447His; C/T	Synonymous polymorphism	C__11922961_30

The general characteristics of genetic polymorphisms tested in our study are shown in Table 3.

5.2.1.1 Analysis of SNPs of SERPINE2, TNF-α and SFTPB genes

The genotype and allele frequencies of SERPINE2, TNF-α and SFTPB by case–control status are given in Table 4. Tests of HWE were carried out for all loci among cases and controls separately; all SNPs were found to be in HWE. Genotype and allele frequencies of the three SNPs in the patients did not differ significantly from those in the control group and none of the previously reported positive association between the analyzed SNPs and COPD was replicated in our study. The exon 3 polymorphism (rs6477096) in SERPINE2 gene showed an elevated odds ratio in an additive logistic regression model adjusted for age, gender and pack-years, however, even this value was not significant (OR = 1,53; 95% CI = 0,85 – 2,80) (Table 4).

Table 4. Allele and genotype frequencies of SERPINE2, TNF-α and SFTPB

Gene Symbol	SNP ID	Allele frequency		Genotype frequency			§Hardy-Weinberg Equilibrium *P*-value	Genetic model	Confindence Interval	*P*-value	Logistic Analysis		Cochran-Armitage trend test (P value)
SERPINE2	rs6747096	A	G	AA (%)	AG (%)	GG (%)		OR [AA] vs [GG]	95% CI		OR	95% CI	*P* Value
Controls		0.719	0.281	143 (49.4)	124 (42.9)	22 (7.6)	0.491	1.484	0.81 - 1.54	0.198	1.53	0.85 - 2.80	0.311
Cases		0.681	0.319	127 (47.0)	114 (42.2)	29 (10.7)	0.652						
TNF-α	rs1800629	G	A	GG (%)	GA (%)	AA (%)		OR [GG] vs [GA + AA]	95% CI		OR	95% CI	
Controls		0.802	0.198	188 (68.1)	89 (30.5)	15 (5.1)	0.299	1.157	0.82 - 1.63	0.403	1.14	0.80 - 1.61	0.628
Cases		0.782	0.218	164 (60.9)	94 (34.9)	11 (4.1)	0.587						
SFTPB	rs1130866	C	T	CC (%)	CT (%)	TT (%)		OR [CC] vs [TT]	95% CI		OR	95% CI	
Controls		0.499	0.501	72 (25.3)	136 (47.9)	76 (26.8)	0.413	1.14	0.72 - 1.81	0.579	1.18	0.79 - 1.75	0.576
Cases		0.471	0.529	64 (23.9)	127 (47.4)	77 (28.7)	0.478						

All of the SNPs were in Hardy-Weinberg equilibrium but did not showed significantly different distribution and odds ratios between COPD and Control group. §χ^2-test was used, OR: odds ratio, CI: confidence interval

5.2.1.2 Analysis of individual SNPs and haplotypes variants of EPHX1 gene

Comparison of the distribution of observed and expected genotypes showed that all EPHX1 SNPs were in HWE in cases and controls. The assessment of the association of individual SNPs with COPD showed a potential trend that homozygosity for the minor allele could increased the risk of disease in case of Tyr113His polymorphism ("slow" allele) (OR= 1.345; 95% CI= 0.96 – 1.91; P= 0.095), while reduced it in case of the His139Arg SNP ("fast" allele) (OR= 0.675; 95% CI= 0.27 – 1.69; P= 0.399). However, none of these SNPs were significantly associated with COPD even after adjusting the model for gender, age and pack-years in logistic regression (Table 5).

Table 5. Allele and genotype frequencies of examined EPHX1 gene polymorphisms

Gene Symbol	SNP ID	Allele frequency		Genotype frequency			§Hardy-Weinberg Equilibrium	OR (95% CI)
		T	C	TT (%)	TC (%)	CC (%)	P value	
Controls	rs1051740	0.723	0.277	154 (53.3)	110 (38.1	25 (8.7)	0.401	1.11 (0.86-1.44)
Cases		0.701	0.299	127 (47.4)	122 (45.5)	19 (7.1)	0.154	
		A	G	AA (%)	AG (%)	GG (%)		
Controls	rs2234922	0.779	0.221	171 (60.0)	102 (35.8)	12 (4.2)	0.507	0.88 (0.66-1.18)
Cases		0.799	0.201	169 (62.8)	92 (64.2)	8 (2.9)	0.280	

§χ^2-test was used, OR: odds ratio, CI: confidence interval

Frequencies for the four SNP based haplotypes were estimated for cases and controls. Although the "slow activity" CA (His^{113}-His^{139}) haplotype was more frequent among cases (24.8% versus the 20.8% in controls), the overall distribution did not differ significantly between the two groups (P= 0.736). Furthermore, alleles of the two loci in EPHX1 are in complete LD as shown by the pair-wise standardized disequilibrium coefficient (D'=0.036).

Due to the presence of these coding variants, marked variations in EPHX1 activity have been reported previously. Therefore we have assessed the association of the predicted (rapid", "normal", "slow" and "very slow") EPHX1 phenotypes with the development of COPD [258, 259]. The distribution of predicted EPHX1 activity was

significantly different between control subjects and COPD patients (P=0.041). In the analysis of predicted phenotypes the COPD group had higher proportion of the predicted "slow" phenotype. Consequently the slow phenotype significantly raises the risk of developing COPD [OR=1.639; 95% CI=1.06–2.49; P=0.021)] in our case control study (Table 6).

Table 6. The distribution of the predicted EPHX1 phenotypes

Predicted EPHX1 activity	Controls n (%)	Cases n (%)	§P value	Contingency tables OR (95% CI)	P value
Normal	144 (53.1)	123 (48.4)	0.041	1 (reference)	
Slow	55 (20.2)	77 (30.3)		1.64 (1.08-2.49)	0.021
Very slow	17 (6.2)	9 (3.5)		0.62 (0.27-1.44)	0.306
Rapid	55 (20.2)	45 (17.7)		0.96 (0.59-3.19)	0.855

Normal: exon3 Tyr/Tyr and exon 4 His/His or exon 3 Tyr/His and exon 4 His/Arg; Slow: exon 3 Tyr/Tyr and exon 4 His/His; Very slow: exon 3 His/His and exon 4 His/His; Rapid: exon 3 Tyr/Tyr and exon 4 Arg/Arg or His/Arg

§χ^2-test was used, OR: odds ratio, CI: confidence interval

5.2.1.3 Analysis of PPARγ in COPD

High expression level of PPARγ mRNA in the lung has been reported previously [260, 261]. We examined PPARγ expression at protein level in surgical lung tissue samples. PPARγ protein is expressed in the lung and most of the PPARγ protein derived signals co-localized with the expression of a typical macrophage marker CD68 and the dendritic cell marker DCSign (Figure 18). mRNA expression of PPARγ is enriched in AM relative to total lung tissue, however we did not find differences in expression level between patients with COPD and healthy individuals (Figure 19). Thus, genetic variants, rather than the level of the mRNA expression of the PPARγ gene could be associated with the development of COPD.

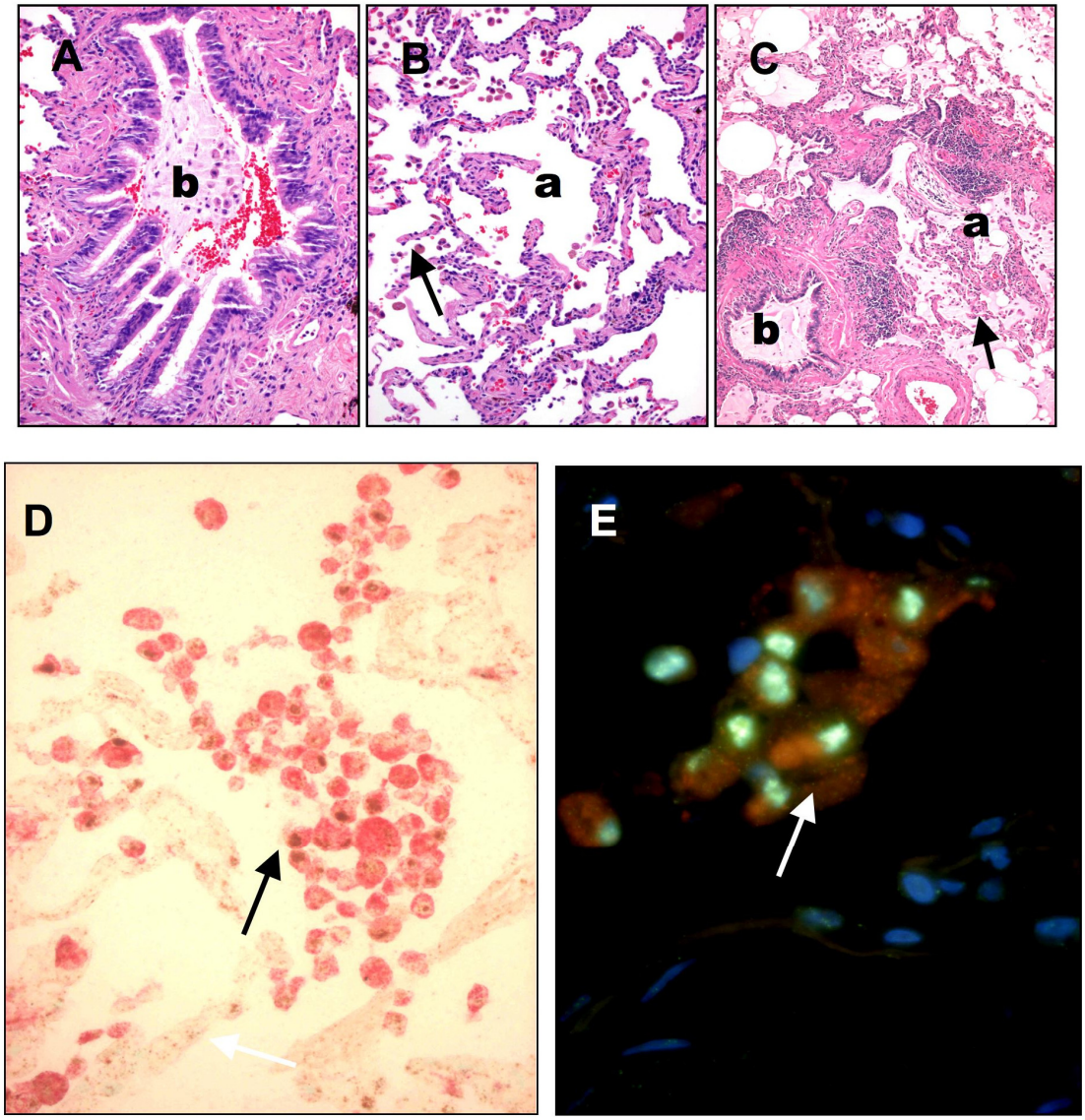


Figure 18. Morphology and immunohistochemistry of COPD-associated lung lesions.

A: Chronic bronchitis.

B: Centriacinar emphysema.

C: Advanced active bronchitis with fibrosis and emphysema.

A-C, hematoxylin-eosin staining;

D: CD68-PPARγ coexpression with double IHC staining using alkaline phosphatase [red cytoplasm-CD68] and diamino-benzidine [brown nuclei-PPARγ].

E: Cells with red fluorescence and green nuclei DCSign-PPARγ double fluorescence staining. Nuclear counter-staining is DAPI.

Indications: **b,** bronchus; **a,** alveolar spaces; **arrows,** alveolar macrophages. *Original magnifications:* **A-D** 20x; **E** 40x;

We have genotyped three exonic SNPs (rs10801282 (Pro12Ala), rs3856806 (His447His) and rs1800571 (Pro113Gln)) in PPARγ gene, but the rs1800571 locus was left out from the analysis because it was homozygous for the major C allele in all individuals. The other two SNPs were in HWE and showed modest LD, the extent of LD between Pro12Ala and His447His was found to be D' = 0.673, although the pair showed lower LD with respect to their correlation coefficient (r^2=0.42).

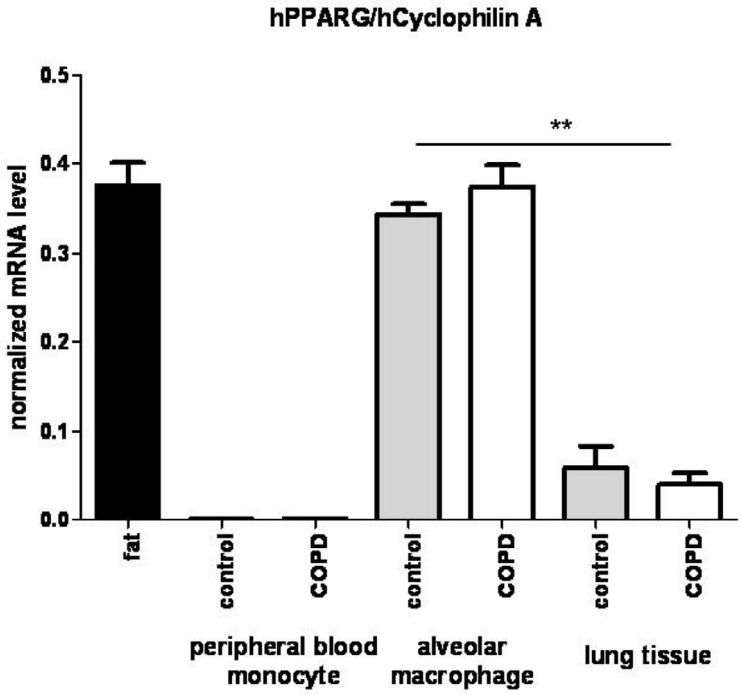


Figure 19. mRNA expression of PPARγ

PPARG showed as high as mRNA expression level in AMs as in subcutan fat and also showed expression in the whole lung tissue with significantly lower level (Mann-Whitney U test). However it was not expressed in peripheral blood MOs.

Data presented normalized values of RT-QPCR measurements; Cyclophilin A was used as housekeeping gene.

Grey bars represent mean values of 5 control patients; white bars represent mean values of 5 COPD patients.

** $p < 0.01$

The single loci allelic and genotypic analysis found no significant association between the two coding variants of PPARγ and COPD. In logistic regression applying a model adjusted for both SNPs, age and pack-years, the rare variant of His447His polymorphism was significantly associated with increased odds for COPD (OR=1.853, 95% CI= 1.09 – 3.14, P=0.021). The minor Ala allele of the Pro12Ala variant had an OR of 0.679, suggesting a protective effect, however it did not reach significance (95% CI= 0.40 – 1.14, P=0.145) (Table 7).

Table 7. Allele and genotype frequencies of examined PPARG gene polymorphisms

Gene Symbol	SNP ID	Allele frequency		Genotype frequency			[§]Hardy-Weinberg Equilibrium	Logistic Analysis	
		C	G	CC (%)	CG (%)	GG (%)	P value	OR (95% CI)	[#]P value
Controls	rs1801282	0.864	0.136	217 (75.2)	64 (22.4)	7 (2.4)	0.398	0.68 (0.40-1.14)	0.15
Cases		0.874	0.126	199 (76.2)	67 (22.3)	4 (1.5)	0.869		
		C	T	CC (%)	CT (%)	TT (%)	P value	OR (95% CI)	[#]P value
Controls	rs3856806	0.882	0.118	224 (78.8)	53 (18.7)	8 (2.5)	0.09	1.85 (1.09-3.14)	0.02
Cases		0.862	0.138	199 (74.0)	65 (24.5)	5 (1.5)	0.57		

[§]χ^2-test was used, OR: odds ratio, CI: confidence interval
[#]Adjusted for age and pack-year

We have estimated the frequency of the possible two-SNP haplotypes of PPARγ and assessed the association between haplotypes and COPD development. There was a significant difference in the frequency of the GC haplotype involving the rare G variant of the Pro12Ala locus between the two groups (P= 0.035). The strength of association was also assessed, for this haplotype (OR= 0.512; 95% CI=0.27–0.96). This finding suggests a protective effect for the GC (12Ala/447His) haplotype of the PPARγ gene for COPD outcome (Table 8).

Table 8. The distribution and strength of association of PPARγ haplotypes

Haplotypes	COPD (freq.)	Control (freq.)	[§]P value	OR (95% CI)
CC	0.834	0.832	0.914	1.02 (0.74-1.40)
CT	0.039	0.030	0.424	1.31 (0.68-2.50)
GC	0.028	0.053	0.035	0.51 (0.27-0.96)
GT	0.098	0.085	0.429	1.18 (0.78-1.77)

[§]χ^2-test was used, OR: odds ratio, CI: confidence interval

5.2.2 IBD SNP STUDY

5.2.2.1 Disease association with individual SNPs: single locus analysis

CD and UC patients were recruited from four centers of Hungarian IBD Study Group. 572 CD, 103 UC and 486 healthy control patients were examined in the study. The diagnosis and the criteria of clinical parameters were according to ECCO Report (Table 9).

Table 9. Clinical parameters of IBD patients

	CD (n=572)	UC (n=103)
Male/Female	254/318	48/55
Mean age (years)	35.6 ± 12.7	41.5 ± 15.5
Age at presentation (years)	28.4 ± 11.3	31.5 ± 14.0
Mean duration (years)	7.9 ± 6.7	10.1 ± 11.5
Disease location (n)		
	L1 (124)	Proctitis (12)
	L2 (146)	Left-sided (48)
	L3 (265)	Extensive (43)
	L4 only (12)	
	L4 (5)	
Disease behavior (n)		
B1	253	
B2	139	-
B3	99	
B4	81	
Perianal disease	145 (25.3%)	-
Frequent relapse	183 (31.9%)	33 (32%)
Arthritis	151 (26.3%)	24 (23.3%)
Ocular manifestation	40 (6.9%)	2 (1.9%)
Cutaneous manifestation	46 (8%)	3 (2.9%)
Steroid use/refractory	397 (69.4%) / 32 (5.6%)	69 (66.9%) / 17 (16.5%)
Immunosupressives	255 (44.5%)	6 (5.8%)
Surgery/multiple in CD	212 (37%) / 50 (8.7%)	8 (7.7%9
Smoking habits		
never	323	83
yes	178	16
past	71	4

Data presented as mean ±SD

Four SNPs of PPARγ were tested; three of them localized in the first intron and the fourth the Pro12Ala in the exon B (Table 10).

Table 10. Information of examined SNPs

SNP code	Nucleotide change	Contig position	Description	TaqMan assay code
rs10865710	C/G	12293198	intron 1 (C-681G)	C___9384417_10
rs2067819	G/A	12299049	intron 1	C__15860374_10
rs3892175	G/A	12308038	intron 1	C___8756599_10
rs1801282	C/G	12333125	exonB (Pro12Ala)	C_26856791_20

Tests of HWE were carried out among CD, UC and control groups separately for all loci and all polymorphisms were found to be in equilibrium in all three groups. The allele and genotype frequencies did not differ significantly among the cohorts. Although the frequency of the rare GG genotype of the Pro12Ala polymorphism occurred with lower frequencies in CD patients compared to controls, this distribution was still not significant (P = 0.088). We would like to note here that despite the difference in the size of the CD and UC cohort, the allele frequencies of the genotyped SNPs did not differ significantly between these groups (Table 11 and Table 12).

Table 11. Allele and genotype frequencies in CD patients

SNP ID		Allele frequency		Genotype frequency			P value	Hardy-Weinberg Equilibrium (P value)
rs10865710 (C-681G)		C	G	CC (%)	CG (%)	GG (%)		
	Controls	0.725	0.275	257 (54.0)	176 (37.0)	43 (9.0)	0.856	0.110
	Cases	0.729	0.271	298 (53.9)	210 (37.0)	45 (9.1)		0.353
rs2067819		G	A	GG (%)	GA (%)	AA (%)		
	Controls	0.761	0.239	280 (58.1)	174 (36.1)	28 (5.8)	0.895	0.887
	Cases	0.898	0.102	331 (59.4)	196 (35.2)	30 (5.4)		0.888
rs3892175		G	A	GG (%)	GA (%)	AA (%)		
	Controls	0.895	0.105	377 (80.2)	87 (18.5)	6 (1.3)	0.957	0.700
	Cases	0.898	0.102	427 (80.9)	94 (17.8)	7 (1.3)		0.483
rs1801282 (Pro12Ala)		C	G	CC (%)	CG (%)	GG (%)		
	Controls	0.868	0.132	375 (76.2)	104 (21.1)	13 (2.6)	0.088	0.082
	Cases	0.881	0.119	433 (77.0)	124 (22.1)	5 (0.9)		0.230

Table 12. Allele and genotype frequencies in UC patients

SNP ID		Allele frequency		Genotype frequency			P value	Hardy-Weinberg Equilibrium (P value)
		C	G	CC (%)	CG (%)	GG (%)		
rs10865710	Controls	0.725	0.275	257 (54.0)	176 (37.0)	43 (9.0)	0.223	0.110
	Cases	0.713	0.287	49 (48.5)	46 (45.5)	6 (5.9)		0.259
		G	A	GG (%)	GA (%)	AA (%)		
rs2067819	Controls	0.761	0.239	280 (58.1)	174 (36.1)	28 (5.8)	0.715	0.887
	Cases	0.767	0.233	58 (57.4)	39 (38.6)	4 (4.0)		0.413
		G	A	GG (%)	GA (%)	AA (%)		
rs3892175	Controls	0.895	0.105	377 (80.2)	87 (18.5)	6 (1.3)	0.525	0.700
	Cases	0.903	0.097	79 (80.6)	19 (19.4)	0 (0.0)		0.287
		C	G	CC (%)	CG (%)	GG (%)		
rs1801282	Controls	0.868	0.132	375 (76.2)	104 (21.1)	13 (2.6)	0.829	0.082
	Cases	0.857	0.143	77 (74.8)	24 (23.3)	2 (1.9)		0.935

When the distribution of individuals carrying the rare Ala/Ala genotype were compared with Pro/Pro homozygotes between CD cases and controls, the association was found to be significant (OR = 0.33; 95%CI = 0.12 – 0.94; P = 0.03), suggesting that the rare Ala allele confers a reduced risk of CD, when homozygous. However we did not find any significant associations of Pro12Ala genotypes with the disease location in CD patients (Table 13).

Table 13. Association of Pro12Ala genotypes with *Disease location* parameter in CD patients

	Pro12Ala			
	Pro/Pro n (%)	Pro/Ala n (%)	Ala/Ala n (%)	[§]P value
L1	90 (76.3%)	26 (22.0%)	2 (1.7%)	0.411
L2	111 (78.7%)	29 (20.6%)	1 (0.7%)	
L3	196 (76.6%)	58 (22.6%)	2 (0.8%)	
L4	9 (75.0%)	3 (25.0%)	0 (0%)	

[§]χ^2-test was used

In contrast, no significant statistical association was detected between the PPARγ Pro12Ala and UC susceptibility. No significant association was detected between the other three SNPs and CD phenotypes.

5.2.2.2 Disease association with SNP haplotypes

To determine the non-random association of alleles on the four loci in PPARγ, pair-wise LD was measured among the four polymorphisms in the CD and UC samples. Figure 20 shows the extent of LD between each pair of all SNPs (the D' values are

given). LD analysis revealed that the four SNPs were in strong LD in both patient groups (all D' values are higher than 0.77). According to the r^2 values, rs10865710 and rs2067819 are in the strongest LD (in the UC/control group $r^2 = 0.697$; in the CD/control group $r^2 = 0.731$).

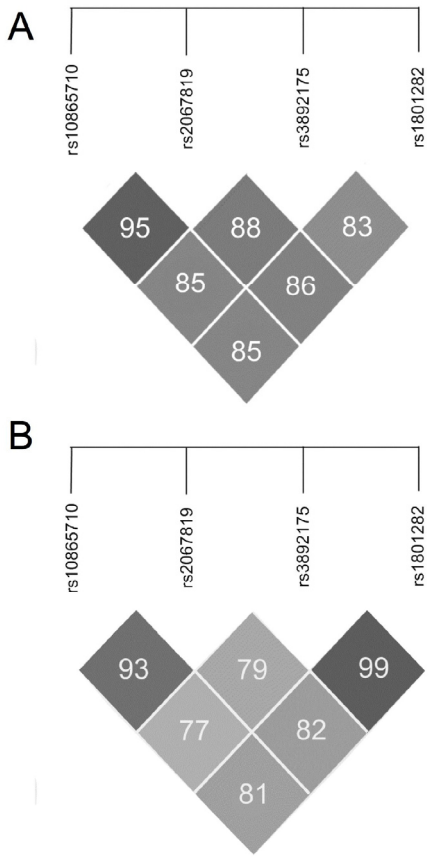


Figure 20. Pairwise linkage disequilibrium results

A. CD cohort

B. UC cohort

The standardized *D' is* shown for each pair of SNPs.

Since genetic association analysis based on haplotypes considered more powerful than single SNP analysis, we have assessed the association between haplotypes and CD or UC. The distributions of the five most frequent four-SNP haplotypes for both diseased groups versus control are shown in Table 14.

Although the allele frequencies did not differ significantly between the CD and UC groups, we observed that the overall frequencies of haplotypes were significantly different between the control and either CD or UC patients (CD/control $P = 3.58 \times 10^{-6}$; UC/Control $P = 2.24 \times 10^{-29}$) and between the two diseased groups, too ($P < 10^{-4}$).

In case of the CD cohort, the only haplotype that showed significant association to the disease was the GAGG haplotype containing the rare Ala variant of the Pro12Ala polymorphism (OR = 0.72, 95% CI: 0.53 – 0.97; P = 0.028, ($P_{corrected}$= 0.11), it confers a protective effect with borderline significance. The presence of the common C allele in the haplotype (GAGC) has an opposite effect, it somewhat increases the risk of CD, however the association is not significant (OR = 1.33; 95% CI: 0.69 – 2.47; P = 0.415).

In case of the UC group three haplotypes showed significant association with the development of the disease. Interestingly, the most common four-SNP haplotype, CGGC was found to be strongly associated with UC, it has a protective effect due to its significantly higher frequency in controls compared to cases (OR = 0.62, 95% CI: 0.44 – 0.87, P = 0.005). After Bonferroni correction for multiple testing (5 haplotypes), the difference observed for haplotypes remained significant ($P_{corrected} = 0.025$). The GAGG haplotype like in the case of CD has a protective effect, too (OR = 0.14; 95% CI: 0.05 – 0.42; $P = 3.78 \times 10^{-5}$, after Bonferroni correction $P_{corrected} = 1.89 \times 10^{-4}$). However, having the common C allele of the Pro12Ala variant instead of the rare G allele – resulting the GAGC haplotype – significantly increases the risk of UC (OR = 6.70; 95% CI: 3.41 – 13.17; $P = 3.85 \times 10^{-10}$, after Bonferroni correction $P_{corrected} = 1.93 \times 10^{-9}$).

Table 14. Estimated haplotype frequencies and association significance and strength in CD and UC patients

	CD/Controls					UC/Controls				
	Frequency (%)		§P value	OR	95% CI	Frequency (%)		§P value	OR	95% CI
	Cases	Control				Cases	Control			
CGGC	70.8	69.9	0.315	1.11	0.90-1.38	57.7	69.9	0.005	0.62	0.44-0.87
GAAC	9.7	8.5	0.317	1.17	0.85-1.61	7.6	8.5	0.772	0.92	0.51-1.65
GAGG	9.0	12.2	0.028	0.72	0.53-0.97	1.9	12.2	3.78×10^{-5}	0.14	0.05-0.42
GAGC	2.6	1.8		nc		10.7	1.8	3.85×10^{-10}	6.7	3.41-13.17
GGGC	4.6	4.9	0.41	0.96	0.62-1.46	6.6	4.9	0.281	1.43	0.75-2.75

§χ^2-test was used, OR: odds ratio, CI: confidence interval, nc: not calculated due to the low frequencies in both populations

6 DISCUSSION

Inflammation is a physiological response to infections and tissue injury, and is part of the immune defense mechanisms, which helps to restore homeostasis at damaged sites. During acute inflammation negative feedback mechanisms are activated such as production of anti-inflammatory cytokines or activation of regulatory cells. Thus, to maintain the healthy homeostasis, the regulation of inflammatory responses is essential. Dysregulated inflammatory responses lead to chronic inflammation, which is believed to underlie the pathophysiolgy of several disorders, can be characterized by loss of barrier function, responsiveness to a normally benign stimulus and elevation of inflammatory cells, overproduction of oxidants, cytokines and chemokines in the involved organs. The increased levels of these factors amplify the inflammatory response, which are destructive and contribute clinical symptoms and progression of the disease [2-4].

Determining disease specific markers and differentiating between disease states are critically important in chronic diseases. As environmental factors and genetic predisposition act together in the development of complex diseases genomic technologies including the microarray and the next generation sequencing technology provides an opportunity to perform global gene expression and genome wide analyses [262]. Several GWAS studies identified disease associated genetic loci and that is proved that the origin or subtypes/stratification of disease condition can be defined by analyzing mRNA expression patterns [263]. Identification of genes with altered expression or marker genes could also be potential targets for novel and more effective therapy [264, 265].

We performed global gene expression analysis of AMs, which are one of the most important cells in the inflammatory process of COPD in our study. We expanded the list a of COPD associated genes, identified and validated list of COPD specific genes which are overlap between AMs and their precursors peripheral MOs and correlated gene expression patterns with lung function parameter [266]. Genetic association studies were also performed and SNPs of an inflammatory transcription regulator gene, PPARγ were examined in association with COPD and IBD.

6.1 Gene expression analysis of COPD

COPD is a prime example of a long-lasting, chronic disease with devastating outcome with essentially no validated clinical or biomarker other than lung function tests available and the Hungarian male population is the most affected in Europe [28, 29]. Previously several studies examined the pathomechanism of COPD at the mRNA level. They analyzed animal models of the disease, performed cell culture experiment and collected primary clinical samples to obtain gene expression data [149-154, 156].

In our study largely independent sets of patients for screen/exploration (microarray) and validation (RT-QPCR) were collected, stringent inclusion/exclusion criteria were determined for the sample collection. Our results suggest that gene sets specific for COPD can be identified in both AMs and MOs based on their expression patterns. Several previously identified COPD related genes such as CYP1B1, PLA2G7, CCL2, SPP1, IL-8 and IL-1β were validated our microarray experiment [150, 151, 154, 156]; in addition we could identify a list of COPD specific probe sets that are expressed differently in diseased and healthy patients in AMs. From this list, 23 genes were confirmed by RT-QPCR on a larger cohort of independent samples and thirteen novel candidate genes were identified, including CCR1, TREM2, GADD45A, PHLDA1 and PHLDA2, EMR3, GDF15, FPRL2, HOMER3, SLC2A3, IGSF6, PLEKHB1 PAPSS2. EMR3 is transmembrane receptor that is expressed on activated macrophages and neutrophils. GADD45A, PHLDA1 and PHLDA2 are connected to cell cycle regulation and apoptosis. TREM2 (triggering receptor) is expressed on macrophages and dendritic cells during immune response. GDF15 (MIC-1) is an early mediator of injury response in several organs (kidney and lung). This protein is a TGF-beta family member with inflammatory cell mediator activity. SLC2A3 is a glucose transporter expressed in early stage of non-small cell lung cancer. IGSF6 is a member of immunoglobulin superfamily. The expression and function in the lung or macrophages of HOMER3, PLEKHB1 are not known.

Elevated level of recruitment of MOs from the blood, which are progenitors of AMs reported in the lungs during progression of COPD. It could be important to evaluate the possibility that there are similar COPD–specific changes in the expression of genes in

the precursor and the differentiated cells. These genes might be used as biomarkers for COPD, which may help in establishing and verifying the diagnosis, during disease progression or regression and in testing the efficacy of treatment. In addition, it would also establish MOs as an affected cell type in COPD.

We identified commonly expressed genes in AM and peripheral MOs. What we consider as a key aspect of our work is the identification and validation of a set of 5 genes showing common expression patterns in AM and MO. It suggests that these two related cell types are both affected in the disease and this is manifested in peripheral gene expression signatures. These genes include PHLDA1, an apoptotic gene that was also validated using AM RNA samples. ADAM10 is a cell surface protein with protease function, activates the inactive form of TNF-α by cleaving. Elevated level of ADAM10 has been previously documented in smokers [154]. IFDR1 is a nervous system specific gene, which could have a role in proliferative and differentiation pathways induced by nerve growth factor. Higher expression level of IFRD1 was observed in lung and other types of cancers. GK is a key enzyme of glycerol metabolism. SEC14L1 is involved in cytosolic transport. The existence of common gene expression pattern in MOs and AMs can be the consequence of different scenarios.

It is possible that both cell types are affected by the disease as the result of a secondary effect due to similar systemic i.e. chronic inflammatory changes resulting in similar alterations. This would also include similar changes in other tissue macrophages. Moreover, similar changes might be found in other chronic inflammatory diseases such as RA, psoriasis or IBD. Our findings suggest that preformed changes in the precursor cell type might have a causative role in the development of the disease and these alterations, either primary or secondary, could influence the differentiated tissue macrophages such as AMs. Both of these possibilities could be tested and their evaluation could lead to important insights into the pathogenesis of COPD and of other chronic inflammatory disorders, including the identification of differentially expressed gene sets and/or markers. It would be particularly intriguing to look at the identified 23 and 5 member gene sets in terms of SNP in order to identify potential genetic changes associated with no only the disease state but clinical parameters. In such studies, regulatory regions SNPs deserve particular attention. Previous studies showed correlation

between several SNPs and accelerated FEV1 decline in COPD [134, 267]. Gene expression signatures were also showed to correlate with clinical parameter. Increased expression level of SPP1 was showed to correlate with decreased lung function in smokers compared smokers, whilst decreased mRNA levels of several antioxidant genes correlated with the degree of airflow limitation [156, 268].

At the same time, simply using the gene sets for validating the hypothesis that peripheral MO gene expression can serve as marker of disease state progression and maybe therapeutic efficacy would also have clinical insights and benefits [264, 269]. Importantly, our results also showed, that the validated COPD-specific gene expression signatures of AMs (CCR1, FN1, GDF15, SLC2A3) and MOs (ADAM10, GK, IFRD1, SEC14L1) correlate with lung function parameter (predicted FEV1%) which may suggest that disease progression can be determined and monitored by analyzing either disease-specific or peripheral blood gene expression patterns using a panel of genes.

6.2 Analyses of SNPs in chronic inflammatory diseases

Beside environmental factors the genetic predisposition plays major role in the development of chronic inflammatory and autoimmune diseases. Identification of disease-susceptible SNPs of candidate genes could be used as primary target, however further validation all of genes are needed. Validation is divided into two types. Validation of association by additional association tests, such as replicate the study on new independent cohort either from the same race or different race. The frequencies of common SNPs could show huge differences between races, which could lead controversial interpretation of disease-susceptibility of SNPs. Validation of the association could be performed by using biological assay. In this case the function of the identified susceptible gene is examined whether this function is relevant to the pathophysiology of disease and disease-associated variant how could be affect this function [9].

6.2.1 PPARγ SUSCEPTIBILITY IN CHRONIC INFLAMMATORY DISEASES

PPARγ has role in negative regulation of inflammatory process, previous studies and clinical trials reported beneficial effect of PPARγ ligand [176, 223, 261]. Based on these findings we investigated PPARγ gene association existed between the presence of certain PPARγ gene polymorphisms and common inflammatory diseases, COPD and IBD [178, 239]. PPARγ highly expressed in the lungs and association of SNPs of PPARγ with the development of asthma has been reported [142]. It plays a role in regulation of gut inflammation; it inhibits the epithelial inflammatory response, it is required for the maintenance of innate antimicrobial immunity in the colon and could be a new promising target in IBD therapy [179].

Polymorphisms of PPARγ have been examined in metabolic syndrome diabetes and obesity, and also in disorders with autoimmune origin such as psoriasis and psoriatic arthritis [270, 271]. However, only a few studies have examined the association of SNPs of PPARγ with in IBD. PPARγ were reported as a susceptibility gene for CD, the minor alleles of two SNPs of intron 1 (rs2067819, rs3892175) were more common in Caucasian controls compared to CD patients, but the allele frequencies of the His447His did not show statistically significant difference [186]. Leung et al. also examined the association between CD and PPARγ and genotyped the Pro12Ala (rs1801282), intron 1 (rs2067819) and the promoter variant C-681G (rs10865710) polymorphisms and found no significant differences between CD patients and controls in a Caucasian population from New Zealand [184]. Atug et al. explored the allele frequencies of Pro12Ala polymorphism in a small Turkish cohort of IBD (69 CD, 45 UC) patients and controls [183]. They found no significant differences between the allele frequencies. In a recent study association of Pro12Ala and His447His SNPs were examined in a Chinese IBD and a white Dutch IBD population. A potential association between His447His polymorphism and UC patients in the Chinese population were found; however this finding could not be replicated in the Dutch population [185].

6.2.2 COPD STUDY

Several polymorphisms were identified as COPD-susceptibility loci, of which gene product functionally might contribute to the pathophysiology of COPD. Although the incidence of COPD shows an increasing rate in Hungary, our study was the first genetic association study, which examined associations between candidate gene polymorphisms and COPD in a Hungarian population. We chose previously tested SNPs, which showed association to COPD in Caucasian cohorts and performed replicate experiment to validate our cohort.

A previous study reported significant association between rs6747096 in exon 3 of SERPINE2 gene and COPD in family-based association study and in a case-control study too. Subsequent case-control studies, however, did not replicate this association in European and Chinese populations [121, 122]. In our study we could not repeat the original result, although rs6747096 seemed to increase the risk of disease (OR= 1.53; 95% CI = 0,85 – 2,80), it did not show significant association [272].

Another candidate gene is TNF-α that codes for a multifunctional pro-inflammatory cytokine. There are conflicting reports about the association to COPD of the –308G>A promoter polymorphism of TNFα gene that is expected to increase the transcriptional activity [273]. In contrast to previous reports about the causative role of this SNP our results did not indicate that TNFα –308G>A significantly increases the risk of COPD [133]. Our finding is supported by the results of recent clinical trials that provided no clinically beneficial effects of systemic anti-TNF-α therapy for COPD patients suggesting that TNFα might not play a central role in the pathophysiology of COPD [272].

Surfactant protein B has an important role in reducing the alveolar surface tension and preventing alveolar collapse at low lung volumes. The non-synonymous Thr113Ile coding variant SNP in SFTPB was found to be significantly associated with COPD in previous case-control studies in Hispanic (OR 3.7, 95% CI 1.2–12.1; p= 0.03) and in a German study [274]. We found no evidence for an association of another previously reported causative variant in SFTPB gene (OR= 1.157 95% CI= 0.72 – 1.81). Not even

an adjustment for age, gender and pack-years in logistic regression analysis elevated the OR to a significant level [272].

EPHX1 is an enzyme associated with the metabolism and detoxification of xenobiotic chemicals; it plays an important role in the general oxidative defense of lung. Several polymorphisms are known in EPHX1 including two relatively common SNPs, the exon 3 Tyr113His and exon 4 His139Arg variants. These two variant alleles have been suggested to be associated with altered EPHX1 enzyme activity. Substitution of Tyr113 for His decreases EPHX1 activity (slow allele), whereas substitution of His139 for Arg increases EPHX1 activity (fast allele). Since EPHX1 is involved in the detoxification of epoxide intermediates in tobacco smoke, any change in the rate of conversion of these highly reactive compounds could affect individual ability to cope with the toxic effect of cigarette smoke [258, 259].

Several studies found the slow metabolizing form of EPHX1 to be associated with an increased risk for COPD so it was a logical candidate gene for our study [275]. In accordance with this tendency, in our study of individual SNPs the homozygous minor Thr113His variant showed borderline association with COPD phenotype (OR= 1.345, 96% CI= 0.96 – 1.91, P= 0.095), however, the level of association was somewhat reduced when the model was adjusted for age, sex and pack-years.

There is complete linkage equilibrium betwen the two analysed SNPs and the distribution of estimated haplotypes did not differ between patient and contol groups. Due to the presence complete linkage equilibrium and HWE for the studied SNPs we reconstructed the frequency of diplotypes and the predicted phenotypes of EPHX1 activity in patient and control groups. The difference in distribution of the predicted phenotypes was significant (P< 0.041) between the two groups. The COPD group had a higher proportion of the predicted slow and lower proportion of predicted normal and rapid EPHX1 activity. Interestingly the control group showed an excess of very slow phenotypes, but its frequency was low in both groups, but the slow phenotype significantly increased the risk for COPD in our study (OR of slow phenotype vs normal = 1.639, 95% CI= 1.06 – 2.49, P= 0.021). This result provides additional support to the notion that EPHX1 is likely to be involved in COPD pathogenesis [272].

IHC staining and RT-QPCR measurements were confirmed at the mRNA and at the protein level that PPARγ is expressed in lung tissues and particular in AM. Although gene expression level did not show difference between patients with COPD and healthy individuals, polymorphisms of this gene still could be potential candidate markers of the disease.

Three SNPs of PPARγ were genotyped – Pro12Ala, His447His and rs1800571 - the rs1800571 was excluded since all individuals carried an identical homozygous genotype. Our single-marker tests for the other two coding variants yielded a significant association for the minor allele of His447His polymorphism in logistic regression adjusted for both SNPs, age, sex and pack-years (OR= 1.853; 95% CI= 1.09-3.14; P= 0.02). However the His447His variant did not cause amino acid change, several papers pointed out that synonymous SNPs can affect mRNA splicing, stability and protein folding. These changes can cause a significant effect on the protein function therefore there is a reason to investigate it further.

A modest pair-wise LD was found between Pro12Ala and His447His. Since the use of SNP-based haplotypes in genetic association studies may offer a more powerful approach than the use of individual SNPs, a haplotype analysis was also performed. A significant difference was found in the frequency of GC haplotype (containing the minor G allele of Pro12Ala and major C for His447His variant) between the control and COPD groups and the association of this haplotype to COPD outcome was also determined. The GC haplotype might contribute to a significant lower risk for COPD, pointing to a potential functional protective effect of this haplotype [272].

We are aware of the fact that significant results could prove to be false positives, and a clear limitation of our study is the relatively low sample size. The study has other limitations such as that population stratification should be investigated in these kinds of studies and we did not analyze a second cohort to replicate our results. Possible gene-gene and gene-environment interactions pose a difficulty for genetic analysis of COPD association studies, too. Further studies using larger populations are needed and other variants in the PPARγ gene should be investigated in order to clarify the association of PPARγ and individual susceptibility to the development of COPD.

6.2.3 IDB STUDY

Since the incidence of IBD is rapidly increasing in Eastern Europe including Hungary and reached the level found in Western European countries, we felt important to study genetic risk factors that might influence susceptibility to IBD in the Eastern European population. Although only limited data are available from this part of Europe, Hungary has a large and well-characterized cohort of IBD patients. In previous studies, several IBD associated SNPs such as polymorphisms in NOD2/CARD15, TLR4, ATG16L1 and IL-23R genes were genotyped and their contributions to susceptibility to IBD were examined of this cohort. Serological markers were also identified and their association to the disease severity combined with the genotype data of NOD2/CARD15 and TLR4 were investigated [276-279].

We have further analyzed an already well-characterized Hungarian IBD cohort extending the characterization of genetic factors by genotyping four SNPs (C-681G, rs2067819, rs3892175 and Pro12Ala) in a potential candidate gene, PPARγ. These SNPs were examined previously in different IBD studies. We did not find any significant differences between CD/UC patients and controls in the case of the allele frequency of C-681G, rs2067819 and rs3892175 polymorphisms. Interestingly, the homozygous variant of the minor allele (Ala/Ala) of Pro12Ala was found to be significantly less common in CD patients compared to controls, confirming significant protective effect compared to homozygous common genotype (OR = 0.33; 95%CI = 0.12 – 0.94; P = 0.03). Strong linkage disequilibrium was identified between the four examined loci (in the pair-wise comparisons all D' values were higher than 0.77) and the associations of potential haplotypes were investigated. The GAGG haplotype, containing the rare Ala variant showed higher frequency in controls compared to CD and also UC patients, suggesting a potential protective effect of this variant against the development of IBD (OR = 0.72, 95% CI: 0.53 – 0.97; $P_{corrected}$= 0.11, OR = 0.14; 95% CI: 0.05 – 0.42; P = 3.78×10^{-5}, $P_{corrected} = 1.89 \times 10^{-4}$.in case of CD and UC cohorts, respectively). In contrast, the GAGC haplotype was significantly more common in UC patients than in healthy controls, increasing the probability of UC phenotype (OR = 6.70; 95% CI: 3.41 – 13.17; P = 3.85×10^{-10}, $P_{corrected} = 1.93 \times 10^{-9}$). Surprisingly the combination of the four major alleles was

significantly more common in UC patients than in controls (CGGC has a protective effect, OR = 0.62, 95% CI: 0.44 – 0.87, P = 0.005, $P_{corrected}$ = 0.025).

We are aware of the fact that our study has some limitations such as the relatively low sample size; and that the population stratification was not investigated. Further studies using larger populations are needed in a different cohort to replicate our results. Other variants in the PPARγ gene should be investigated as well in order to clarify the association of PPARγ and individual susceptibility to the development of IBD.

7 CONCLUSIONS

We used genomic and genetic analyses to characterize chronic inflammatory diseases. We performed gene expression analysis and sought to define gene sets showing differential expression patterns between healthy controls and patients with COPD. Gene expression patterns of AMs and peripheral MOs were examined in order to evaluate whether the disease affects either or both cell types and if there is an overlap in altered gene expression. We identified and validated COPD-specific overlapping signatures of expression profiles of peripheral blood MOs and AMs demonstrating that peripheral MOs are also affected by COPD. The discriminating power of the set of 23 genes validated in AM samples; and the set of 5 genes validated in MO samples were also demonstrated by using CVA that could separate COPD and healthy patients in both cases in which the χ^2 test of canonical correlation was significant at $p= 0.05$, suggesting that the gene lists do have strong discriminating power even though separation of the two groups was not perfect. We found that some of the validated expression changes correlate with lung function parameter FEV1%. The presented data can provide a basis for more mechanistic studies of disease pathomechanisms and some of the differentially expressed genes might serve as biomarkers of disease progression and effectiveness of therapy.

We performed the first genetic association study of Hungarian patients with COPD. In a replicate study five SNPs of four genes were genotyped, which previously were reported as COPD-associated polymorphisms in Caucasian population. This experiment validated our cohort, however we could not found association to COPD in the case of SERPINE2, TNF-α and SFTPB, but our study provided support for the suggested causative role of EPHX1 polymorphisms and phenotypes imputed from exon 3 and exon 4 genotype data in COPD outcome in a Hungarian population.

Further we have carried out the first investigation of PPARγ gene polymorphisms in a case-control COPD study and characterized the association between individual SNPs and haplotypes in PPARγ and susceptibility to COPD. Although the GC haplotype has a modest protective effect, it might point toward the potential importance of common alleles with weak effect in heterogeneous diseases, like COPD. Documentation of PPARγ

haplotype association with COPD identifies this important gene as a target of further investigation for the pathogenesis of COPD and as a potential target of therapy.

In addition we have investigated four polymorphisms of PPARγ in a well-characterized Hungarian IBD cohort. This is the first study that examines the association between PPARγ and IBD in Eastern Europe. We found association between IBD and PPARγ at single loci level suggesting a potential protective effect of the minor Ala allele in CD and we also found haplotypes, which have significantly different frequency between CD/UC patients and controls. However we could not replicate previous results, where the minor alleles of the two non-coding polymorphisms in intron 1 (rs2067819 and rs3892175) were found to have significantly higher frequencies in CD cases than in controls. Nonetheless these data provide further support for the notion that a nuclear hormone receptor is linked to human intestinal inflammation and warrant further studies on the exact mechanism contributing to disease progression and also as a potential therapeutic target.

8 ACKNOWLEDGEMENTS

I would like to thank my supervisor Prof. László Nagy for helping and guiding my work.
I would like to thank István Szatmári, Attila Szántó, Lajos Széles, Tamás Varga, Bertalan Meskó, Zsolt Czimmerer and Zoltán Simándi for their comments on my manuscripts and thesis.
I would like to thank the technical assistance of Ibolya Fürtös and Márta Béládi.
I thank all of my colleagues in the Nuclear Hormone Receptor research laboratory and in the Department of Biochemistry and Molecular Biology for their help.
I also would like to thank all of our collaborators for their help and excellent work. I would like to thank László Takács from BioSystems International, Iain Kilty from Pfizer András Penyige from Department of Human Genetics, Mária Papp and Károly Palatka from 2nd Department of Medicine for helping to prepare the manuscripts and Balázs Dezső from Department of Pathology for performing immunohistochemistry.
Thanks for the clinical coordination to colleagues of the Department of Pulmonolgy: Eszter Csánky, Zsuzsa Bodnár, Attila Vaskó, Péter Szabó, Sándor Sz. Kiss, Titanilla Tölgyesi, Mária Ráduly, and to all of the members of the Hungarian IBD Study group: Semmelweis University, 1st Department of Medicine, Budapest: Simon Fischer, Henrik Csaba Horvath, Lajos S Kiss; Csolnoky F. County Hospital, 1st Department of Medicine, Veszprém: Zsuzsanna Erdelyi, Gabor Mester, Tunde Pandur; Erzsebet Hospital, 1st Department of Medicine, Budapest: Agota Kovacs, Laszlo Bene; University of Szeged, 1st Department of Medicine, Szeged: Ferenc Nagy, Klaudia Farkas; University of Debrecen, 2nd Department of Medicine, Debrecen: Istvan Altorjay, Zsuzsanna Vitalis; Semmelweis University, 2nd Department of Medicine, Semmelweis University, Budapest: Pal Miheller, Zsolt Tulassay, Laszlo Herszenyi for the help with clinical sample collection and analysis.

9 REFERENCES

1. Eder W, Ege MJ, von Mutius E. The asthma epidemic. *N Engl J Med* 2006: 355(21): 2226-2235.
2. Renz H, von Mutius E, Brandtzaeg P, Cookson WO, Autenrieth IB, Haller D. Gene-environment interactions in chronic inflammatory disease. *Nat Immunol*: 12(4): 273-277.
3. Nathan C. Points of control in inflammation. *Nature* 2002: 420(6917): 846-852.
4. Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, Folkerts G, Friedmann PS, Frost GS, Guarner F, Lovik M, Macfarlane S, Meyer PD, M'Rabet L, Serafini M, van Eden W, van Loo J, Vas Dias W, Vidry S, Winklhofer-Roob BM, Zhao J. Inflammatory disease processes and interactions with nutrition. *Br J Nutr* 2009: 101 Suppl 1: S1-45.
5. Zhang W, Dolan ME. Beyond the HapMap Genotypic Data: Prospects of Deep Resequencing Projects. *Curr Bioinform* 2008: 3(3): 178.
6. Zhang W, Dolan ME. Impact of the 1000 genomes project on the next wave of pharmacogenomic discovery. *Pharmacogenomics*: 11(2): 249-256.
7. Durbin RM, Abecasis GR, Altshuler DL, Auton A, Brooks LD, Gibbs RA, Hurles ME, McVean GA. A map of human genome variation from population-scale sequencing. *Nature*: 467(7319): 1061-1073.
8. Heath SC, Gut IG, Brennan P, McKay JD, Bencko V, Fabianova E, Foretova L, Georges M, Janout V, Kabesch M, Krokan HE, Elvestad MB, Lissowska J, Mates D, Rudnai P, Skorpen F, Schreiber S, Soria JM, Syvanen AC, Meneton P, Hercberg S, Galan P, Szeszenia-Dabrowska N, Zaridze D, Genin E, Cardon LR, Lathrop M. Investigation of the fine structure of European populations with applications to disease association studies. *Eur J Hum Genet* 2008: 16(12): 1413-1429.
9. Yamada R, Ymamoto K. Recent findings on genes associated with inflammatory disease. *Mutat Res* 2005: 573(1-2): 136-151.
10. Hardy J, Singleton A. Genomewide association studies and human disease. *N Engl J Med* 2009: 360(17): 1759-1768.
11. Hunter DJ, Kraft P. Drinking from the fire hose--statistical issues in genomewide association studies. *N Engl J Med* 2007: 357(5): 436-439.
12. Wang WY, Barratt BJ, Clayton DG, Todd JA. Genome-wide association studies: theoretical and practical concerns. *Nat Rev Genet* 2005: 6(2): 109-118.
13. Capon F, Allen MH, Ameen M, Burden AD, Tillman D, Barker JN, Trembath RC. A synonymous SNP of the corneodesmosin gene leads to increased mRNA stability and demonstrates association with psoriasis across diverse ethnic groups. *Hum Mol Genet* 2004: 13(20): 2361-2368.
14. Sauna ZE, Kimchi-Sarfaty C, Ambudkar SV, Gottesman MM. Silent polymorphisms speak: how they affect pharmacogenomics and the treatment of cancer. *Cancer Res* 2007: 67(20): 9609-9612.
15. Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, Aach J, Ansorge W, Ball CA, Causton HC, Gaasterland T, Glenisson P, Holstege FC, Kim IF, Markowitz V, Matese JC, Parkinson H, Robinson A, Sarkans U, Schulze-Kremer S, Stewart J, Taylor R, Vilo J, Vingron M. Minimum information about a microarray

experiment (MIAME)-toward standards for microarray data. *Nat Genet* 2001: 29(4): 365-371.
16. Enkemann SA. Standards affecting the consistency of gene expression arrays in clinical applications. *Cancer Epidemiol Biomarkers Prev*: 19(4): 1000-1003.
17. Baechler EC, Batliwalla FM, Reed AM, Peterson EJ, Gaffney PM, Moser KL, Gregersen PK, Behrens TW. Gene expression profiling in human autoimmunity. *Immunol Rev* 2006: 210: 120-137.
18. Heller RA, Schena M, Chai A, Shalon D, Bedilion T, Gilmore J, Woolley DE, Davis RW. Discovery and analysis of inflammatory disease-related genes using cDNA microarrays. *Proc Natl Acad Sci U S A* 1997: 94(6): 2150-2155.
19. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JI, Yang L, Marti GE, Moore T, Hudson J, Jr., Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Levy R, Wilson W, Grever MR, Byrd JC, Botstein D, Brown PO, Staudt LM. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000: 403(6769): 503-511.
20. Sotiriou C, Wirapati P, Loi S, Harris A, Fox S, Smeds J, Nordgren H, Farmer P, Praz V, Haibe-Kains B, Desmedt C, Larsimont D, Cardoso F, Peterse H, Nuyten D, Buyse M, Van de Vijver MJ, Bergh J, Piccart M, Delorenzi M. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst* 2006: 98(4): 262-272.
21. van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002: 415(6871): 530-536.
22. Talbot SG, Estilo C, Maghami E, Sarkaria IS, Pham DK, P Oc, Socci ND, Ngai I, Carlson D, Ghossein R, Viale A, Park BJ, Rusch VW, Singh B. Gene expression profiling allows distinction between primary and metastatic squamous cell carcinomas in the lung. *Cancer Res* 2005: 65(8): 3063-3071.
23. Lequerre T, Gauthier-Jauneau AC, Bansard C, Derambure C, Hiron M, Vittecoq O, Daveau M, Mejjad O, Daragon A, Tron F, Le Loet X, Salier JP. Gene profiling in white blood cells predicts infliximab responsiveness in rheumatoid arthritis. *Arthritis Res Ther* 2006: 8(4): R105.
24. Schadt EE, Friend SH, Shaywitz DA. A network view of disease and compound screening. *Nat Rev Drug Discov* 2009: 8(4): 286-295.
25. Glocker MO, Guthke R, Kekow J, Thiesen HJ. Rheumatoid arthritis, a complex multifactorial disease: on the way toward individualized medicine. *Med Res Rev* 2006: 26(1): 63-87.
26. Barnes PJ. Mediators of chronic obstructive pulmonary disease. *Pharmacol Rev* 2004: 56(4): 515-548.
27. Lopez AD, Murray CC. The global burden of disease, 1990-2020. *Nat Med* 1998: 4(11): 1241-1243.
28. Hurd SS. International efforts directed at attacking the problem of COPD. *Chest* 2000: 117(5 Suppl 2): 336S-338S.
29. Hurd S. The impact of COPD on lung health worldwide: epidemiology and incidence. *Chest* 2000: 117(2 Suppl): 1S-4S.

30. Barnes PJ. New treatments for chronic obstructive pulmonary disease. *Ann Ist Super Sanita* 2003: 39(4): 573-582.
31. Barnes PJ. Therapy of chronic obstructive pulmonary disease. *Pharmacol Ther* 2003: 97(1): 87-94.
32. Barnes PJ. Mechanisms in COPD: differences from asthma. *Chest* 2000: 117(2 Suppl): 10S-14S.
33. Chung KF. Cytokines in chronic obstructive pulmonary disease. *Eur Respir J Suppl* 2001: 34: 50s-59s.
34. Maestrelli P, Paska C, Saetta M, Turato G, Nowicki Y, Monti S, Formichi B, Miniati M, Fabbri LM. Decreased haem oxygenase-1 and increased inducible nitric oxide synthase in the lung of severe COPD patients. *Eur Respir J* 2003: 21(6): 971-976.
35. Corrigan CJ, Kay AB. The roles of inflammatory cells in the pathogenesis of asthma and of chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1991: 143(5 Pt 1): 1165-1168; discussion 1175-1166.
36. Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. *J Clin Invest* 2008: 118(11): 3546-3556.
37. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol* 2008: 8(3): 183-192.
38. Cazzola M, Matera MG, Rogliani P, Page C. Treating systemic effects of COPD. *Trends Pharmacol Sci* 2007: 28(10): 544-550.
39. Turato G, Zuin R, Saetta M. Pathogenesis and pathology of COPD. *Respiration* 2001: 68(2): 117-128.
40. Mannino DM. Epidemiology and global impact of chronic obstructive pulmonary disease. *Semin Respir Crit Care Med* 2005: 26(2): 204-210.
41. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet* 2004: 364(9435): 709-721.
42. Barnes PJ. Chronic obstructive pulmonary disease. *N Engl J Med* 2000: 343(4): 269-280.
43. Barnes PJ. New treatments for COPD. *Nat Rev Drug Discov* 2002: 1(6): 437-446.
44. MacNee W. Pathogenesis of chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2005: 2(4): 258-266; discussion 290-251.
45. Cosio MG, Guerassimov A. Chronic obstructive pulmonary disease. Inflammation of small airways and lung parenchyma. *Am J Respir Crit Care Med* 1999: 160(5 Pt 2): S21-25.
46. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, Pare PD. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004: 350(26): 2645-2653.
47. Reilly JJ. COPD and declining FEV1--time to divide and conquer? *N Engl J Med* 2008: 359(15): 1616-1618.
48. Papi A, Luppi F, Franco F, Fabbri LM. Pathophysiology of exacerbations of chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2006: 3(3): 245-251.
49. Celli BR. Predictors of mortality in COPD. *Respir Med*: 104(6): 773-779.
50. Scanlon PD, Connett JE, Waller LA, Altose MD, Bailey WC, Buist AS. Smoking cessation and lung function in mild-to-moderate chronic obstructive pulmonary disease. The Lung Health Study. *Am J Respir Crit Care Med* 2000: 161(2 Pt 1): 381-390.

51. Yoshida T, Tuder RM. Pathobiology of cigarette smoke-induced chronic obstructive pulmonary disease. *Physiol Rev* 2007: 87(3): 1047-1082.
52. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. *Eur Respir J* 2009: 33(5): 1165-1185.
53. Sin DD, Wu L, Man SF. The relationship between reduced lung function and cardiovascular mortality: a population-based study and a systematic review of the literature. *Chest* 2005: 127(6): 1952-1959.
54. Gan WQ, Man SF, Sin DD. The interactions between cigarette smoking and reduced lung function on systemic inflammation. *Chest* 2005: 127(2): 558-564.
55. Barreiro E, de la Puente B, Minguella J, Corominas JM, Serrano S, Hussain SN, Gea J. Oxidative stress and respiratory muscle dysfunction in severe chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2005: 171(10): 1116-1124.
56. Tockman MS, Anthonisen NR, Wright EC, Donithan MG. Airways obstruction and the risk for lung cancer. *Ann Intern Med* 1987: 106(4): 512-518.
57. Wasswa-Kintu S, Gan WQ, Man SF, Pare PD, Sin DD. Relationship between reduced forced expiratory volume in one second and the risk of lung cancer: a systematic review and meta-analysis. *Thorax* 2005: 60(7): 570-575.
58. Barnes PJ. New concepts in chronic obstructive pulmonary disease. *Annu Rev Med* 2003: 54: 113-129.
59. Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. *Eur Respir J* 2003: 22(4): 672-688.
60. Di Stefano A, Caramori G, Ricciardolo FL, Capelli A, Adcock IM, Donner CF. Cellular and molecular mechanisms in chronic obstructive pulmonary disease: an overview. *Clin Exp Allergy* 2004: 34(8): 1156-1167.
61. Lacoste JY, Bousquet J, Chanez P, Van Vyve T, Simony-Lafontaine J, Lequeu N, Vic P, Enander I, Godard P, Michel FB. Eosinophilic and neutrophilic inflammation in asthma, chronic bronchitis, and chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 1993: 92(4): 537-548.
62. Di Stefano A, Maestrelli P, Roggeri A, Turato G, Calabro S, Potena A, Mapp CE, Ciaccia A, Covacev L, Fabbri LM, et al. Upregulation of adhesion molecules in the bronchial mucosa of subjects with chronic obstructive bronchitis. *Am J Respir Crit Care Med* 1994: 149(3 Pt 1): 803-810.
63. Traves SL, Culpitt SV, Russell RE, Barnes PJ, Donnelly LE. Increased levels of the chemokines GROalpha and MCP-1 in sputum samples from patients with COPD. *Thorax* 2002: 57(7): 590-595.
64. Terashima T, Wiggs B, English D, Hogg JC, van Eeden SF. Phagocytosis of small carbon particles (PM10) by alveolar macrophages stimulates the release of polymorphonuclear leukocytes from bone marrow. *Am J Respir Crit Care Med* 1997: 155(4): 1441-1447.
65. Terashima T, Wiggs B, English D, Hogg JC, van Eeden SF. The effect of cigarette smoking on the bone marrow. *Am J Respir Crit Care Med* 1997: 155(3): 1021-1026.
66. Richards GA, Theron AJ, Van der Merwe CA, Anderson R. Spirometric abnormalities in young smokers correlate with increased chemiluminescence responses of activated blood phagocytes. *Am Rev Respir Dis* 1989: 139(1): 181-187.

67. Stanescu D, Sanna A, Veriter C, Kostianev S, Calcagni PG, Fabbri LM, Maestrelli P. Airways obstruction, chronic expectoration, and rapid decline of FEV1 in smokers are associated with increased levels of sputum neutrophils. *Thorax* 1996: 51(3): 267-271.
68. Finkelstein R, Fraser RS, Ghezzo H, Cosio MG. Alveolar inflammation and its relation to emphysema in smokers. *Am J Respir Crit Care Med* 1995: 152(5 Pt 1): 1666-1672.
69. Majo J, Ghezzo H, Cosio MG. Lymphocyte population and apoptosis in the lungs of smokers and their relation to emphysema. *Eur Respir J* 2001: 17(5): 946-953.
70. Saetta M, Turato G, Maestrelli P, Mapp CE, Fabbri LM. Cellular and structural bases of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001: 163(6): 1304-1309.
71. Saetta M, Baraldo S, Turato G, Beghe B, Casoni GL, Bellettato CM, Rea F, Zuin R, Fabbri LM, Papi A. Increased proportion of CD8+ T-lymphocytes in the paratracheal lymph nodes of smokers with mild COPD. *Sarcoidosis Vasc Diffuse Lung Dis* 2003: 20(1): 28-32.
72. Vukmanovic-Stejic M, Vyas B, Gorak-Stolinska P, Noble A, Kemeny DM. Human Tc1 and Tc2/Tc0 CD8 T-cell clones display distinct cell surface and functional phenotypes. *Blood* 2000: 95(1): 231-240.
73. Saetta M, Mariani M, Panina-Bordignon P, Turato G, Buonsanti C, Baraldo S, Bellettato CM, Papi A, Corbetta L, Zuin R, Sinigaglia F, Fabbri LM. Increased expression of the chemokine receptor CXCR3 and its ligand CXCL10 in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2002: 165(10): 1404-1409.
74. Retamales I, Elliott WM, Meshi B, Coxson HO, Pare PD, Sciurba FC, Rogers RM, Hayashi S, Hogg JC. Amplification of inflammation in emphysema and its association with latent adenoviral infection. *Am J Respir Crit Care Med* 2001: 164(3): 469-473.
75. van der Strate BW, Postma DS, Brandsma CA, Melgert BN, Luinge MA, Geerlings M, Hylkema MN, van den Berg A, Timens W, Kerstjens HA. Cigarette smoke-induced emphysema: A role for the B cell? *Am J Respir Crit Care Med* 2006: 173(7): 751-758.
76. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. *Annu Rev Immunol* 2000: 18: 767-811.
77. Lommatzsch M, Bratke K, Bier A, Julius P, Kuepper M, Luttmann W, Virchow JC. Airway dendritic cell phenotypes in inflammatory diseases of the human lung. *Eur Respir J* 2007: 30(5): 878-886.
78. D'Hulst A I, Maes T, Bracke KR, Demedts IK, Tournoy KG, Joos GF, Brusselle GG. Cigarette smoke-induced pulmonary emphysema in scid-mice. Is the acquired immune system required? *Respir Res* 2005: 6: 147.
79. Demedts IK, Bracke KR, Van Pottelberge G, Testelmans D, Verleden GM, Vermassen FE, Joos GF, Brusselle GG. Accumulation of dendritic cells and increased CCL20 levels in the airways of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2007: 175(10): 998-1005.
80. Bracke K, Cataldo D, Maes T, Gueders M, Noel A, Foidart JM, Brusselle G, Pauwels RA. Matrix metalloproteinase-12 and cathepsin D expression in pulmonary

macrophages and dendritic cells of cigarette smoke-exposed mice. *Int Arch Allergy Immunol* 2005: 138(2): 169-179.
81. Smelter DF, Sathish V, Thompson MA, Pabelick CM, Vassallo R, Prakash YS. Thymic stromal lymphopoietin in cigarette smoke-exposed human airway smooth muscle. *J Immunol*: 185(5): 3035-3040.
82. Floreani AA, Wyatt TA, Stoner J, Sanderson SD, Thompson EG, Allen-Gipson D, Heires AJ. Smoke and C5a induce airway epithelial intercellular adhesion molecule-1 and cell adhesion. *Am J Respir Cell Mol Biol* 2003: 29(4): 472-482.
83. Hellermann GR, Nagy SB, Kong X, Lockey RF, Mohapatra SS. Mechanism of cigarette smoke condensate-induced acute inflammatory response in human bronchial epithelial cells. *Respir Res* 2002: 3: 22.
84. Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, Okada Y, Yamasawa F, Nakahara K, Umeda A. Increased expression of transforming growth factor-beta1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med* 2001: 163(6): 1476-1483.
85. Adler KB, Li Y. Airway epithelium and mucus: intracellular signaling pathways for gene expression and secretion. *Am J Respir Cell Mol Biol* 2001: 25(4): 397-400.
86. Demoly P, Simony-Lafontaine J, Chanez P, Pujol JL, Lequeux N, Michel FB, Bousquet J. Cell proliferation in the bronchial mucosa of asthmatics and chronic bronchitics. *Am J Respir Crit Care Med* 1994: 150(1): 214-217.
87. Franklin WA, Veve R, Hirsch FR, Helfrich BA, Bunn PA, Jr. Epidermal growth factor receptor family in lung cancer and premalignancy. *Semin Oncol* 2002: 29(1 Suppl 4): 3-14.
88. Shapiro SD. The macrophage in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999: 160(5 Pt 2): S29-32.
89. Tetley TD. Macrophages and the pathogenesis of COPD. *Chest* 2002: 121(5 Suppl): 156S-159S.
90. Frankenberger M, Menzel M, Betz R, Kassner G, Weber N, Kohlhaufl M, Haussinger K, Ziegler-Heitbrock L. Characterization of a population of small macrophages in induced sputum of patients with chronic obstructive pulmonary disease and healthy volunteers. *Clin Exp Immunol* 2004: 138(3): 507-516.
91. Capelli A, Di Stefano A, Gnemmi I, Balbo P, Cerutti CG, Balbi B, Lusuardi M, Donner CF. Increased MCP-1 and MIP-1beta in bronchoalveolar lavage fluid of chronic bronchitics. *Eur Respir J* 1999: 14(1): 160-165.
92. Marques LJ, Teschler H, Guzman J, Costabel U. Smoker's lung transplanted to a nonsmoker. Long-term detection of smoker's macrophages. *Am J Respir Crit Care Med* 1997: 156(5): 1700-1702.
93. Di Stefano A, Capelli A, Lusuardi M, Balbo P, Vecchio C, Maestrelli P, Mapp CE, Fabbri LM, Donner CF, Saetta M. Severity of airflow limitation is associated with severity of airway inflammation in smokers. *Am J Respir Crit Care Med* 1998: 158(4): 1277-1285.
94. Hirani N, Antonicelli F, Strieter RM, Wiesener MS, Ratcliffe PJ, Haslett C, Donnelly SC. The regulation of interleukin-8 by hypoxia in human macrophages--a potential role in the pathogenesis of the acute respiratory distress syndrome (ARDS). *Mol Med* 2001: 7(10): 685-697.

95. Culpitt SV, Rogers DF, Shah P, De Matos C, Russell RE, Donnelly LE, Barnes PJ. Impaired inhibition by dexamethasone of cytokine release by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2003: 167(1): 24-31.
96. Punturieri A, Filippov S, Allen E, Caras I, Murray R, Reddy V, Weiss SJ. Regulation of elastinolytic cysteine proteinase activity in normal and cathepsin K-deficient human macrophages. *J Exp Med* 2000: 192(6): 789-799.
97. Russell RE, Thorley A, Culpitt SV, Dodd S, Donnelly LE, Demattos C, Fitzgerald M, Barnes PJ. Alveolar macrophage-mediated elastolysis: roles of matrix metalloproteinases, cysteine, and serine proteases. *Am J Physiol Lung Cell Mol Physiol* 2002: 283(4): L867-873.
98. Finlay GA, Russell KJ, McMahon KJ, D'Arcy E M, Masterson JB, FitzGerald MX, O'Connor CM. Elevated levels of matrix metalloproteinases in bronchoalveolar lavage fluid of emphysematous patients. *Thorax* 1997: 52(6): 502-506.
99. Russell RE, Culpitt SV, DeMatos C, Donnelly L, Smith M, Wiggins J, Barnes PJ. Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2002: 26(5): 602-609.
100. Demedts IK, Brusselle GG, Bracke KR, Vermaelen KY, Pauwels RA. Matrix metalloproteinases in asthma and COPD. *Curr Opin Pharmacol* 2005: 5(3): 257-263.
101. Finlay GA, O'Driscoll LR, Russell KJ, D'Arcy EM, Masterson JB, FitzGerald MX, O'Connor CM. Matrix metalloproteinase expression and production by alveolar macrophages in emphysema. *Am J Respir Crit Care Med* 1997: 156(1): 240-247.
102. Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science* 1997: 277(5334): 2002-2004.
103. Smith SF, Roberts NA, Guz A, Tetley TD. Compromised inhibition of human lung lavage cell elastases. *FEBS Lett* 1996: 390(2): 187-190.
104. Campbell EJ, White RR, Senior RM, Rodriguez RJ, Kuhn C. Receptor-mediated binding and internalization of leukocyte elastase by alveolar macrophages in vitro. *J Clin Invest* 1979: 64(3): 824-833.
105. MacNee W. Oxidants/antioxidants and COPD. *Chest* 2000: 117(5 Suppl 1): 303S-317S.
106. Joos L, Pare PD, Sandford AJ. Genetic risk factors of chronic obstructive pulmonary disease. *Swiss Med Wkly* 2002: 132(3-4): 27-37.
107. Sandford AJ, Joos L, Pare PD. Genetic risk factors for chronic obstructive pulmonary disease. *Curr Opin Pulm Med* 2002: 8(2): 87-94.
108. Castaldi PJ, Cho MH, Cohn M, Langerman F, Moran S, Tarragona N, Moukhachen H, Venugopal R, Hasimja D, Kao E, Wallace B, Hersh CP, Bagade S, Bertram L, Silverman EK, Trikalinos TA. The COPD genetic association compendium: a comprehensive online database of COPD genetic associations. *Hum Mol Genet*: 19(3): 526-534.
109. Malfait R, Gorus F, Sevens C. Electrophoresis of serum protein to detect alpha 1-antitrypsin deficiency: five illustrative cases. *Clin Chem* 1985: 31(8): 1397-1399.

110. Nukiwa T, Brantly ML, Ogushi F, Fells GA, Crystal RG. Characterization of the gene and protein of the common alpha 1-antitrypsin normal M2 allele. *Am J Hum Genet* 1988: 43(3): 322-330.
111. Brantly ML, Paul LD, Miller BH, Falk RT, Wu M, Crystal RG. Clinical features and history of the destructive lung disease associated with alpha-1-antitrypsin deficiency of adults with pulmonary symptoms. *Am Rev Respir Dis* 1988: 138(2): 327-336.
112. Ogushi F, Hubbard RC, Fells GA, Casolaro MA, Curiel DT, Brantly ML, Crystal RG. Evaluation of the S-type of alpha-1-antitrypsin as an in vivo and in vitro inhibitor of neutrophil elastase. *Am Rev Respir Dis* 1988: 137(2): 364-370.
113. Segura-Valdez L, Pardo A, Gaxiola M, Uhal BD, Becerril C, Selman M. Upregulation of gelatinases A and B, collagenases 1 and 2, and increased parenchymal cell death in COPD. *Chest* 2000: 117(3): 684-694.
114. Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ, Brinckerhoff CE. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res* 1998: 58(23): 5321-5325.
115. Joos L, He JQ, Shepherdson MB, Connett JE, Anthonisen NR, Pare PD, Sandford AJ. The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. *Hum Mol Genet* 2002: 11(5): 569-576.
116. Lee SY, Kim MJ, Kang HG, Yoo SS, Choi YY, Lee WK, Cha SI, Kim CH, Jung TH, Park JY. Polymorphisms in matrix metalloproteinase-1, -9 and -12 genes and the risk of chronic obstructive pulmonary disease in a Korean population. *Respiration*: 80(2): 133-138.
117. Ito I, Nagai S, Handa T, Muro S, Hirai T, Tsukino M, Mishima M. Matrix metalloproteinase-9 promoter polymorphism associated with upper lung dominant emphysema. *Am J Respir Crit Care Med* 2005: 172(11): 1378-1382.
118. Zhou M, Huang SG, Wan HY, Li B, Deng WW, Li M. Genetic polymorphism in matrix metalloproteinase-9 and the susceptibility to chronic obstructive pulmonary disease in Han population of south China. *Chin Med J (Engl)* 2004: 117(10): 1481-1484.
119. Silverman EK, Mosley JD, Palmer LJ, Barth M, Senter JM, Brown A, Drazen JM, Kwiatkowski DJ, Chapman HA, Campbell EJ, Province MA, Rao DC, Reilly JJ, Ginns LC, Speizer FE, Weiss ST. Genome-wide linkage analysis of severe, early-onset chronic obstructive pulmonary disease: airflow obstruction and chronic bronchitis phenotypes. *Hum Mol Genet* 2002: 11(6): 623-632.
120. Scott RW, Bergman BL, Bajpai A, Hersh RT, Rodriguez H, Jones BN, Barreda C, Watts S, Baker JB. Protease nexin. Properties and a modified purification procedure. *J Biol Chem* 1985: 260(11): 7029-7034.
121. Demeo DL, Mariani TJ, Lange C, Srisuma S, Litonjua AA, Celedon JC, Lake SL, Reilly JJ, Chapman HA, Mecham BH, Haley KJ, Sylvia JS, Sparrow D, Spira AE, Beane J, Pinto-Plata V, Speizer FE, Shapiro SD, Weiss ST, Silverman EK. The SERPINE2 gene is associated with chronic obstructive pulmonary disease. *Am J Hum Genet* 2006: 78(2): 253-264.
122. Chappell S, Daly L, Morgan K, Baranes TG, Roca J, Rabinovich R, Millar A, Donnelly SC, Keatings V, MacNee W, Stolk J, Hiemstra PS, Miniati M, Monti S, O'Connor CM, Kalsheker N. The SERPINE2 gene and chronic obstructive pulmonary disease. *Am J Hum Genet* 2006: 79(1): 184-186; author reply 186-187.

123. Hunninghake GW, Crystal RG. Cigarette smoking and lung destruction. Accumulation of neutrophils in the lungs of cigarette smokers. *Am Rev Respir Dis* 1983: 128(5): 833-838.
124. Wood AM, Stockley RA. The genetics of chronic obstructive pulmonary disease. *Respir Res* 2006: 7: 130.
125. Cosma G, Crofts F, Taioli E, Toniolo P, Garte S. Relationship between genotype and function of the human CYP1A1 gene. *J Toxicol Environ Health* 1993: 40(2-3): 309-316.
126. Cantlay AM, Lamb D, Gillooly M, Norrman J, Morrison D, Smith CA, Harrison DJ. Association between the CYP1A1 gene polymorphism and susceptibility to emphysema and lung cancer. *Clin Mol Pathol* 1995: 48(4): M210-214.
127. Hassett C, Aicher L, Sidhu JS, Omiecinski CJ. Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. *Hum Mol Genet* 1994: 3(3): 421-428.
128. Hersh CP, Demeo DL, Lange C, Litonjua AA, Reilly JJ, Kwiatkowski D, Laird N, Sylvia JS, Sparrow D, Speizer FE, Weiss ST, Silverman EK. Attempted replication of reported chronic obstructive pulmonary disease candidate gene associations. *Am J Respir Cell Mol Biol* 2005: 33(1): 71-78.
129. Hersh CP, Demeo DL, Lazarus R, Celedon JC, Raby BA, Reilly JJ, Silverman EK. Genetic determinants of functional impairment in chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2006: 3(6): 476.
130. Hersh CP, Demeo DL, Lazarus R, Celedon JC, Raby BA, Benditt JO, Criner G, Make B, Martinez FJ, Scanlon PD, Sciurba FC, Utz JP, Reilly JJ, Silverman EK. Genetic association analysis of functional impairment in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2006: 173(9): 977-984.
131. Kiyohara C, Yoshimasu K, Takayama K, Nakanishi Y. EPHX1 polymorphisms and the risk of lung cancer: a HuGE review. *Epidemiology* 2006: 17(1): 89-99.
132. Hersh CP, DeMeo DL, Reilly JJ, Silverman EK. Xenobiotic metabolizing enzyme gene polymorphisms predict response to lung volume reduction surgery. *Respir Res* 2007: 8: 59.
133. Brogger J, Steen VM, Eiken HG, Gulsvik A, Bakke P. Genetic association between COPD and polymorphisms in TNF, ADRB2 and EPHX1. *Eur Respir J* 2006: 27(4): 682-688.
134. van Diemen CC, Postma DS, Vonk JM, Bruinenberg M, Nolte IM, Boezen HM. Decorin and TGF-beta1 polymorphisms and development of COPD in a general population. *Respir Res* 2006: 7: 89.
135. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, Carter ND, Spector TD. Genetic control of the circulating concentration of transforming growth factor type beta1. *Hum Mol Genet* 1999: 8(1): 93-97.
136. Suthanthiran M, Li B, Song JO, Ding R, Sharma VK, Schwartz JE, August P. Transforming growth factor-beta 1 hyperexpression in African-American hypertensives: A novel mediator of hypertension and/or target organ damage. *Proc Natl Acad Sci U S A* 2000: 97(7): 3479-3484.
137. Ishii T, Matsuse T, Teramoto S, Matsui H, Miyao M, Hosoi T, Takahashi H, Fukuchi Y, Ouchi Y. Neither IL-1beta, IL-1 receptor antagonist, nor TNF-alpha

polymorphisms are associated with susceptibility to COPD. *Respir Med* 2000: 94(9): 847-851.
138. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W, Auwerx J. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 1998: 20(3): 284-287.
139. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 2000: 26(1): 76-80.
140. Landi S, Moreno V, Gioia-Patricola L, Guino E, Navarro M, de Oca J, Capella G, Canzian F. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. *Cancer Res* 2003: 63(13): 3560-3566.
141. Jiang J, Gajalakshmi V, Wang J, Kuriki K, Suzuki S, Nakamura S, Akasaka S, Ishikawa H, Tokudome S. Influence of the C161T but not Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma on colorectal cancer in an Indian population. *Cancer Sci* 2005: 96(8): 507-512.
142. Oh SH, Park SM, Lee YH, Cha JY, Lee JY, Shin EK, Park JS, Park BL, Shin HD, Park CS. Association of peroxisome proliferator-activated receptor-gamma gene polymorphisms with the development of asthma. *Respir Med* 2009: 103(7): 1020-1024.
143. Oh SH, Park SM, Park JS, Jang AS, Lee YM, Uh ST, Kim YH, Choi IS, Kim MK, Park BL, Shin HD, Park CS. Association analysis of peroxisome proliferator-activated receptors gamma gene polymorphisms with asprin hypersensitivity in asthmatics. *Allergy Asthma Immunol Res* 2009: 1(1): 30-35.
144. Palmer CN, Doney AS, Ismail T, Lee SP, Murrie I, Macgregor DF, Mukhopadhyay S. PPARG locus haplotype variation and exacerbations in asthma. *Clin Pharmacol Ther* 2007: 81(5): 713-718.
145. Hizawa N, Makita H, Nasuhara Y, Betsuyaku T, Itoh Y, Nagai K, Hasegawa M, Nishimura M. Beta2-adrenergic receptor genetic polymorphisms and short-term bronchodilator responses in patients with COPD. *Chest* 2007: 132(5): 1485-1492.
146. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. *J Clin Invest* 1997: 100(12): 3184-3188.
147. Kim WJ, Hoffman E, Reilly J, Hersh C, Demeo D, Washko G, Silverman EK. Association of COPD candidate genes with computed tomography emphysema and airway phenotypes in severe COPD. *Eur Respir J*: 37(1): 39-43.
148. Bhattacharya S, Srisuma S, Demeo DL, Reilly JJ, Bueno R, Silverman EK, Mariani TJ. Microarray data-based prioritization of chronic obstructive pulmonary disease susceptibility genes. *Proc Am Thorac Soc* 2006: 3(6): 472.
149. Golpon HA, Coldren CD, Zamora MR, Cosgrove GP, Moore MD, Tuder RM, Geraci MW, Voelkel NF. Emphysema lung tissue gene expression profiling. *Am J Respir Cell Mol Biol* 2004: 31(6): 595-600.
150. Spira A, Beane J, Pinto-Plata V, Kadar A, Liu G, Shah V, Celli B, Brody JS. Gene expression profiling of human lung tissue from smokers with severe emphysema. *Am J Respir Cell Mol Biol* 2004: 31(6): 601-610.

151. Ning W, Li CJ, Kaminski N, Feghali-Bostwick CA, Alber SM, Di YP, Otterbein SL, Song R, Hayashi S, Zhou Z, Pinsky DJ, Watkins SC, Pilewski JM, Sciurba FC, Peters DG, Hogg JC, Choi AM. Comprehensive gene expression profiles reveal pathways related to the pathogenesis of chronic obstructive pulmonary disease. *Proc Natl Acad Sci U S A* 2004: 101(41): 14895-14900.
152. Hackett NR, Heguy A, Harvey BG, O'Connor TP, Luettich K, Flieder DB, Kaplan R, Crystal RG. Variability of antioxidant-related gene expression in the airway epithelium of cigarette smokers. *Am J Respir Cell Mol Biol* 2003: 29(3 Pt 1): 331-343.
153. Harvey BG, Heguy A, Leopold PL, Carolan BJ, Ferris B, Crystal RG. Modification of gene expression of the small airway epithelium in response to cigarette smoking. *J Mol Med* 2007: 85(1): 39-53.
154. Heguy A, O'Connor TP, Luettich K, Worgall S, Cieciuch A, Harvey BG, Hackett NR, Crystal RG. Gene expression profiling of human alveolar macrophages of phenotypically normal smokers and nonsmokers reveals a previously unrecognized subset of genes modulated by cigarette smoking. *J Mol Med* 2006: 84(4): 318-328.
155. Kent L, Smyth L, Clayton C, Scott L, Cook T, Stephens R, Fox S, Hext P, Farrow S, Singh D. Cigarette smoke extract induced cytokine and chemokine gene expression changes in COPD macrophages. *Cytokine* 2008: 42(2): 205-216.
156. Woodruff PG, Koth LL, Yang YH, Rodriguez MW, Favoreto S, Dolganov GM, Paquet AC, Erle DJ. A distinctive alveolar macrophage activation state induced by cigarette smoking. *Am J Respir Crit Care Med* 2005: 172(11): 1383-1392.
157. Shanahan F. Crohn's disease. *Lancet* 2002: 359(9300): 62-69.
158. Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002: 347(6): 417-429.
159. Podolsky DK. The current future understanding of inflammatory bowel disease. *Best Pract Res Clin Gastroenterol* 2002: 16(6): 933-943.
160. Sands BE. Inflammatory bowel disease: past, present, and future. *J Gastroenterol* 2007: 42(1): 16-25.
161. Ince MN, Elliott DE. Immunologic and molecular mechanisms in inflammatory bowel disease. *Surg Clin North Am* 2007: 87(3): 681-696.
162. Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, Bamba T, Fujiyama Y. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut* 2003: 52(1): 65-70.
163. Andoh A, Ogawa A, Bamba S, Fujiyama Y. Interaction between interleukin-17-producing CD4+ T cells and colonic subepithelial myofibroblasts: what are they doing in mucosal inflammation? *J Gastroenterol* 2007: 42 Suppl 17: 29-33.
164. Liu Z, Yadav PK, Xu X, Su J, Chen C, Tang M, Lin H, Yu J, Qian J, Yang PC, Wang X. The increased expression of IL-23 in inflammatory bowel disease promotes intraepithelial and lamina propria lymphocyte inflammatory responses and cytotoxicity. *J Leukoc Biol*: 89(4): 597-606.
165. Van Limbergen J, Russell RK, Nimmo ER, Satsangi J. The genetics of inflammatory bowel disease. *Am J Gastroenterol* 2007: 102(12): 2820-2831.
166. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007: 448(7152): 427-434.
167. Massey D, Parkes M. Common pathways in Crohn's disease and other inflammatory diseases revealed by genomics. *Gut* 2007: 56(11): 1489-1492.

168. Bonen DK, Cho JH. The genetics of inflammatory bowel disease. *Gastroenterology* 2003: 124(2): 521-536.
169. Prescott NJ, Fisher SA, Franke A, Hampe J, Onnie CM, Soars D, Bagnall R, Mirza MM, Sanderson J, Forbes A, Mansfield JC, Lewis CM, Schreiber S, Mathew CG. A nonsynonymous SNP in ATG16L1 predisposes to ileal Crohn's disease and is independent of CARD15 and IBD5. *Gastroenterology* 2007: 132(5): 1665-1671.
170. Roberts RL, Gearry RB, Hollis-Moffatt JE, Miller AL, Reid J, Abkevich V, Timms KM, Gutin A, Lanchbury JS, Merriman TR, Barclay ML, Kennedy MA. IL23R R381Q and ATG16L1 T300A are strongly associated with Crohn's disease in a study of New Zealand Caucasians with inflammatory bowel disease. *Am J Gastroenterol* 2007: 102(12): 2754-2761.
171. Tremelling M, Cummings F, Fisher SA, Mansfield J, Gwilliam R, Keniry A, Nimmo ER, Drummond H, Onnie CM, Prescott NJ, Sanderson J, Bredin F, Berzuini C, Forbes A, Lewis CM, Cardon L, Deloukas P, Jewell D, Mathew CG, Parkes M, Satsangi J. IL23R variation determines susceptibility but not disease phenotype in inflammatory bowel disease. *Gastroenterology* 2007: 132(5): 1657-1664.
172. Peyrin-Biroulet L, Beisner J, Wang G, Nuding S, Oommen ST, Kelly D, Parmentier-Decrucq E, Dessein R, Merour E, Chavatte P, Grandjean T, Bressenot A, Desreumaux P, Colombel JF, Desvergne B, Stange EF, Wehkamp J, Chamaillard M. Peroxisome proliferator-activated receptor gamma activation is required for maintenance of innate antimicrobial immunity in the colon. *Proc Natl Acad Sci U S A*: 107(19): 8772-8777.
173. Saez E, Tontonoz P, Nelson MC, Alvarez JG, Ming UT, Baird SM, Thomazy VA, Evans RM. Activators of the nuclear receptor PPARgamma enhance colon polyp formation. *Nat Med* 1998: 4(9): 1058-1061.
174. Sarraf P, Mueller E, Jones D, King FJ, DeAngelo DJ, Partridge JB, Holden SA, Chen LB, Singer S, Fletcher C, Spiegelman BM. Differentiation and reversal of malignant changes in colon cancer through PPARgamma. *Nat Med* 1998: 4(9): 1046-1052.
175. Ponferrada A, Caso JR, Alou L, Colon A, Sevillano D, Moro MA, Lizasoain I, Menchen P, Gomez-Lus ML, Lorenzo P, Cos E, Leza JC, Menchen L. The role of PPARgamma on restoration of colonic homeostasis after experimental stress-induced inflammation and dysfunction. *Gastroenterology* 2007: 132(5): 1791-1803.
176. Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, Flanigan A, Murthy S, Lazar MA, Wu GD. A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. *J Clin Invest* 1999: 104(4): 383-389.
177. Desreumaux P, Dubuquoy L, Nutten S, Peuchmaur M, Englaro W, Schoonjans K, Derijard B, Desvergne B, Wahli W, Chambon P, Leibowitz MD, Colombel JF, Auwerx J. Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor gamma (PPARgamma) heterodimer. A basis for new therapeutic strategies. *J Exp Med* 2001: 193(7): 827-838.
178. Dubuquoy L, Rousseaux C, Thuru X, Peyrin-Biroulet L, Romano O, Chavatte P, Chamaillard M, Desreumaux P. PPARgamma as a new therapeutic target in inflammatory bowel diseases. *Gut* 2006: 55(9): 1341-1349.
179. Rousseaux C, Lefebvre B, Dubuquoy L, Lefebvre P, Romano O, Auwerx J, Metzger D, Wahli W, Desvergne B, Naccari GC, Chavatte P, Farce A, Bulois P, Cortot

A, Colombel JF, Desreumaux P. Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor-gamma. *J Exp Med* 2005: 201(8): 1205-1215.
180. Wada K, Kamisaki Y. [Anti-inflammatory effect of PPARgamma agonists: basics and clinical applications]. *Nippon Rinsho*: 68(2): 278-283.
181. Liang HL, Ouyang Q. [A clinical trial of rosiglitazone and 5-aminosalicylate combination for ulcerative colitis]. *Zhonghua Nei Ke Za Zhi* 2006: 45(7): 548-551.
182. Aoyagi Y, Nagata S, Kudo T, Fujii T, Wada M, Chiba Y, Ohtsuka Y, Yamashiro Y, Shimizu T, Ohkusa T. Peroxisome proliferator-activated receptor gamma 2 mutation may cause a subset of ulcerative colitis. *Pediatr Int*: 52(5): 729-734.
183. Atug O, Tahan V, Eren F, Tiftikci A, Imeryuz N, Hamzaoglu HO, Tozun N. Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma (PPARgamma) gene in inflammatory bowel disease. *J Gastrointestin Liver Dis* 2008: 17(4): 433-437.
184. Leung E, Hong J, Fraser AG, Merriman TR, Vishnu P, Krissansen GW. Peroxisome proliferator-activated receptor-gamma gene polymorphisms and Crohn's disease. *Int J Colorectal Dis* 2007: 22(4): 453-454.
185. Shrestha UK, Karimi O, Crusius JB, Zhou F, Wang Z, Chen Z, van Bodegraven AA, Xiao J, Morre SA, Wang H, Li J, Xia B. Distribution of peroxisome proliferator-activated receptor-gamma polymorphisms in Chinese and Dutch patients with inflammatory bowel disease. *Inflamm Bowel Dis*: 16(2): 312-319.
186. Sugawara K, Olson TS, Moskaluk CA, Stevens BK, Hoang S, Kozaiwa K, Cominelli F, Ley KF, McDuffie M. Linkage to peroxisome proliferator-activated receptor-gamma in SAMP1/YitFc mice and in human Crohn's disease. *Gastroenterology* 2005: 128(2): 351-360.
187. Wang F, Tahara T, Arisawa T, Sakata M, Takahama K, Watanabe M, Hirata I, Nakano H. Polymorphism of peroxisome proliferator-activated receptor gamma is not associated to Japanese ulcerative colitis. *Hepatogastroenterology* 2008: 55(81): 73-75.
188. Xiao W. Advances in NF-kappaB signaling transduction and transcription. *Cell Mol Immunol* 2004: 1(6): 425-435.
189. Jawien J, Gajda M, Mateuszuk L, Olszanecki R, Jakubowski A, Szlachcic A, Korabiowska M, Korbut R. Inhibition of nuclear factor-kappaB attenuates artherosclerosis in apoE/LDLR - double knockout mice. *J Physiol Pharmacol* 2005: 56(3): 483-489.
190. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M. IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 2005: 11(2): 191-198.
191. De Bosscher K, Vanden Berghe W, Haegeman G. The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression. *Endocr Rev* 2003: 24(4): 488-522.
192. Valledor AF, Ricote M. Nuclear receptor signaling in macrophages. *Biochem Pharmacol* 2004: 67(2): 201-212.
193. Hoffmann A, Natoli G, Ghosh G. Transcriptional regulation via the NF-kappaB signaling module. *Oncogene* 2006: 25(51): 6706-6716.
194. Chen L, Fischle W, Verdin E, Greene WC. Duration of nuclear NF-kappaB action regulated by reversible acetylation. *Science* 2001: 293(5535): 1653-1657.

195. Chen LF, Mu Y, Greene WC. Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB. *EMBO J* 2002: 21(23): 6539-6548.
196. Deng WG, Wu KK. Regulation of inducible nitric oxide synthase expression by p300 and p50 acetylation. *J Immunol* 2003: 171(12): 6581-6588.
197. Deng WG, Zhu Y, Wu KK. Up-regulation of p300 binding and p50 acetylation in tumor necrosis factor-alpha-induced cyclooxygenase-2 promoter activation. *J Biol Chem* 2003: 278(7): 4770-4777.
198. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J* 2004: 23(12): 2369-2380.
199. Tomita K, Barnes PJ, Adcock IM. The effect of oxidative stress on histone acetylation and IL-8 release. *Biochem Biophys Res Commun* 2003: 301(2): 572-577.
200. Ito K, Ito M, Elliott WM, Cosio B, Caramori G, Kon OM, Barczyk A, Hayashi S, Adcock IM, Hogg JC, Barnes PJ. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. *N Engl J Med* 2005: 352(19): 1967-1976.
201. Wagner EF, Eferl R. Fos/AP-1 proteins in bone and the immune system. *Immunol Rev* 2005: 208: 126-140.
202. Karin M, Liu Z, Zandi E. AP-1 function and regulation. *Curr Opin Cell Biol* 1997: 9(2): 240-246.
203. Evans RM. The steroid and thyroid hormone receptor superfamily. *Science* 1988: 240(4854): 889-895.
204. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. The nuclear receptor superfamily: the second decade. *Cell* 1995: 83(6): 835-839.
205. Giguere V. Orphan nuclear receptors: from gene to function. *Endocr Rev* 1999: 20(5): 689-725.
206. Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. *Cell* 1995: 83(6): 841-850.
207. Bensinger SJ, Tontonoz P. Integration of metabolism and inflammation by lipid-activated nuclear receptors. *Nature* 2008: 454(7203): 470-477.
208. Glass CK, Ogawa S. Combinatorial roles of nuclear receptors in inflammation and immunity. *Nat Rev Immunol* 2006: 6(1): 44-55.
209. Jonat C, Rahmsdorf HJ, Park KK, Cato AC, Gebel S, Ponta H, Herrlich P. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* 1990: 62(6): 1189-1204.
210. Auphan N, Didonato JA, Helmberg A, Rosette C, Karin M. Immunoregulatory genes and immunosuppression by glucocorticoids. *Arch Toxicol Suppl* 1997: 19: 87-95.
211. Ito K, Chung KF, Adcock IM. Update on glucocorticoid action and resistance. *J Allergy Clin Immunol* 2006: 117(3): 522-543.
212. Ito K, Yamamura S, Essilfie-Quaye S, Cosio B, Ito M, Barnes PJ, Adcock IM. Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF-kappaB suppression. *J Exp Med* 2006: 203(1): 7-13.
213. Ito K, Hanazawa T, Tomita K, Barnes PJ, Adcock IM. Oxidative stress reduces histone deacetylase 2 activity and enhances IL-8 gene expression: role of tyrosine nitration. *Biochem Biophys Res Commun* 2004: 315(1): 240-245.

214. Deeb RS, Hao G, Gross SS, Laine M, Qiu JH, Resnick B, Barbar EJ, Hajjar DP, Upmacis RK. Heme catalyzes tyrosine 385 nitration and inactivation of prostaglandin H2 synthase-1 by peroxynitrite. *J Lipid Res* 2006: 47(5): 898-911.
215. Buchczyk DP, Grune T, Sies H, Klotz LO. Modifications of glyceraldehyde-3-phosphate dehydrogenase induced by increasing concentrations of peroxynitrite: early recognition by 20S proteasome. *Biol Chem* 2003: 384(2): 237-241.
216. Ito K, Lim S, Caramori G, Chung KF, Barnes PJ, Adcock IM. Cigarette smoking reduces histone deacetylase 2 expression, enhances cytokine expression, and inhibits glucocorticoid actions in alveolar macrophages. *FASEB J* 2001: 15(6): 1110-1112.
217. Castrillo A, Tontonoz P. Nuclear receptors in macrophage biology: at the crossroads of lipid metabolism and inflammation. *Annu Rev Cell Dev Biol* 2004: 20: 455-480.
218. Berger J, Moller DE. The mechanisms of action of PPARs. *Annu Rev Med* 2002: 53: 409-435.
219. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 1990: 347(6294): 645-650.
220. Szatmari I, Nagy L. Nuclear receptor signalling in dendritic cells connects lipids, the genome and immune function. *EMBO J* 2008: 27(18): 2353-2362.
221. Spears M, McSharry C, Thomson NC. Peroxisome proliferator-activated receptor-gamma agonists as potential anti-inflammatory agents in asthma and chronic obstructive pulmonary disease. *Clin Exp Allergy* 2006: 36(12): 1494-1504.
222. Asada K, Sasaki S, Suda T, Chida K, Nakamura H. Antiinflammatory roles of peroxisome proliferator-activated receptor gamma in human alveolar macrophages. *Am J Respir Crit Care Med* 2004: 169(2): 195-200.
223. Straus DS, Glass CK. Anti-inflammatory actions of PPAR ligands: new insights on cellular and molecular mechanisms. *Trends Immunol* 2007: 28(12): 551-558.
224. Dreyer C, Keller H, Mahfoudi A, Laudet V, Krey G, Wahli W. Positive regulation of the peroxisomal beta-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). *Biol Cell* 1993: 77(1): 67-76.
225. Cuzzocrea S, Di Paola R, Mazzon E, Genovese T, Muia C, Centorrino T, Caputi AP. Role of endogenous and exogenous ligands for the peroxisome proliferators activated receptors alpha (PPAR-alpha) in the development of inflammatory bowel disease in mice. *Lab Invest* 2004: 84(12): 1643-1654.
226. Oliver WR, Jr., Shenk JL, Snaith MR, Russell CS, Plunket KD, Bodkin NL, Lewis MC, Winegar DA, Sznaidman ML, Lambert MH, Xu HE, Sternbach DD, Kliewer SA, Hansen BC, Willson TM. A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. *Proc Natl Acad Sci U S A* 2001: 98(9): 5306-5311.
227. Chawla A, Lee CH, Barak Y, He W, Rosenfeld J, Liao D, Han J, Kang H, Evans RM. PPARdelta is a very low-density lipoprotein sensor in macrophages. *Proc Natl Acad Sci U S A* 2003: 100(3): 1268-1273.
228. Gallardo-Soler A, Gomez-Nieto C, Campo ML, Marathe C, Tontonoz P, Castrillo A, Corraliza I. Arginase I induction by modified lipoproteins in macrophages: a peroxisome proliferator-activated receptor-gamma/delta-mediated effect that links lipid metabolism and immunity. *Mol Endocrinol* 2008: 22(6): 1394-1402.

229. Meirhaeghe A, Amouyel P. Impact of genetic variation of PPARgamma in humans. *Mol Genet Metab* 2004: 83(1-2): 93-102.
230. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 1995: 83(5): 803-812.
231. Houseknecht KL, Cole BM, Steele PJ. Peroxisome proliferator-activated receptor gamma (PPARgamma) and its ligands: a review. *Domest Anim Endocrinol* 2002: 22(1): 1-23.
232. Shah YM, Morimura K, Gonzalez FJ. Expression of peroxisome proliferator-activated receptor-gamma in macrophage suppresses experimentally induced colitis. *Am J Physiol Gastrointest Liver Physiol* 2007: 292(2): G657-666.
233. Giaginis C, Giagini A, Theocharis S. Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) ligands as potential therapeutic agents to treat arthritis. *Pharmacol Res* 2009: 60(3): 160-169.
234. Honda K, Marquillies P, Capron M, Dombrowicz D. Peroxisome proliferator-activated receptor gamma is expressed in airways and inhibits features of airway remodeling in a mouse asthma model. *J Allergy Clin Immunol* 2004: 113(5): 882-888.
235. Ogawa S, Lozach J, Benner C, Pascual G, Tangirala RK, Westin S, Hoffmann A, Subramaniam S, David M, Rosenfeld MG, Glass CK. Molecular determinants of crosstalk between nuclear receptors and toll-like receptors. *Cell* 2005: 122(5): 707-721.
236. Birrell MA, Patel HJ, McCluskie K, Wong S, Leonard T, Yacoub MH, Belvisi MG. PPAR-gamma agonists as therapy for diseases involving airway neutrophilia. *Eur Respir J* 2004: 24(1): 18-23.
237. Liu D, Zeng BX, Zhang SH, Yao SL. Rosiglitazone, an agonist of peroxisome proliferator-activated receptor gamma, reduces pulmonary inflammatory response in a rat model of endotoxemia. *Inflamm Res* 2005: 54(11): 464-470.
238. Milam JE, Keshamouni VG, Phan SH, Hu B, Gangireddy SR, Hogaboam CM, Standiford TJ, Thannickal VJ, Reddy RC. PPAR-gamma agonists inhibit profibrotic phenotypes in human lung fibroblasts and bleomycin-induced pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2008: 294(5): L891-901.
239. Belvisi MG, Hele DJ. Peroxisome proliferator-activated receptors as novel targets in lung disease. *Chest* 2008: 134(1): 152-157.
240. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007: 356(24): 2457-2471.
241. Diamond GA, Kaul S. Rosiglitazone and cardiovascular risk. *N Engl J Med* 2007: 357(9): 938-939; author reply 939-940.
242. Diamond GA, Bax L, Kaul S. Uncertain effects of rosiglitazone on the risk for myocardial infarction and cardiovascular death. *Ann Intern Med* 2007: 147(8): 578-581.
243. Lakatos PL. Recent trends in the epidemiology of inflammatory bowel diseases: up or down? *World J Gastroenterol* 2006: 12(38): 6102-6108.
244. Lennard-Jones JE. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989: 170: 2-6; discussion 16-19.
245. Stange EF, Travis SP, Vermeire S, Beglinger C, Kupcinkas L, Geboes K, Barakauskiene A, Villanacci V, Von Herbay A, Warren BF, Gasche C, Tilg H, Schreiber SW, Scholmerich J, Reinisch W. European evidence based consensus on the diagnosis

and management of Crohn's disease: definitions and diagnosis. *Gut* 2006: 55 Suppl 1: i1-15.
246. Gasche C, Scholmerich J, Brynskov J, D'Haens G, Hanauer SB, Irvine EJ, Jewell DP, Rachmilewitz D, Sachar DB, Sandborn WJ, Sutherland LR. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflamm Bowel Dis* 2000: 6(1): 8-15.
247. Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus Jr EV, Pena AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005: 19 Suppl A: 5-36.
248. Elias JA, Schreiber AD, Gustilo K, Chien P, Rossman MD, Lammie PJ, Daniele RP. Differential interleukin 1 elaboration by unfractionated and density fractionated human alveolar macrophages and blood monocytes: relationship to cell maturity. *J Immunol* 1985: 135(5): 3198-3204.
249. Bacsi K, Kosa JP, Lazary A, Balla B, Horvath H, Kis A, Nagy Z, Takacs I, Lakatos P, Speer G. LCT 13910 C/T polymorphism, serum calcium, and bone mineral density in postmenopausal women. *Osteoporos Int* 2009: 20(4): 639-645.
250. Balla B, Kosa JP, Kiss J, Podani J, Takacs I, Lazary A, Nagy Z, Bacsi K, Speer G, Lakatos P. Transcriptional profiling of immune system-related genes in postmenopausal osteoporotic versus non-osteoporotic human bone tissue. *Clin Immunol* 2009: 131(2): 354-359.
251. Gogolak P, Rethi B, Szatmari I, Lanyi A, Dezso B, Nagy L, Rajnavolgyi E. Differentiation of CD1a- and CD1a+ monocyte-derived dendritic cells is biased by lipid environment and PPARgamma. *Blood* 2007: 109(2): 643-652.
252. Chiano MN, Clayton DG. Genotypic relative risks under ordered restriction. *Genet Epidemiol* 1998: 15(2): 135-146.
253. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res* 2005: 15(2): 97-98.
254. Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, He L, Shi Y. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). *Cell Res* 2009: 19(4): 519-523.
255. Shapiro SD, Ingenito EP. The pathogenesis of chronic obstructive pulmonary disease: advances in the past 100 years. *Am J Respir Cell Mol Biol* 2005: 32(5): 367-372.
256. Caruso M, Holgate ST, Polosa R. Adenosine signalling in airways. *Curr Opin Pharmacol* 2006: 6(3): 251-256.
257. Wang H, Liu X, Umino T, Skold CM, Zhu Y, Kohyama T, Spurzem JR, Romberger DJ, Rennard SI. Cigarette smoke inhibits human bronchial epithelial cell repair processes. *Am J Respir Cell Mol Biol* 2001: 25(6): 772-779.
258. Benhamou S, Reinikainen M, Bouchardy C, Dayer P, Hirvonen A. Association between lung cancer and microsomal epoxide hydrolase genotypes. *Cancer Res* 1998: 58(23): 5291-5293.

259. Smith CA, Harrison DJ. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. *Lancet* 1997: 350(9078): 630-633.
260. Huang TH, Razmovski-Naumovski V, Kota BP, Lin DS, Roufogalis BD. The pathophysiological function of peroxisome proliferator-activated receptor-gamma in lung-related diseases. *Respir Res* 2005: 6: 102.
261. Belvisi MG, Hele DJ, Birrell MA. Peroxisome proliferator-activated receptor gamma agonists as therapy for chronic airway inflammation. *Eur J Pharmacol* 2006: 533(1-3): 101-109.
262. Dixon AL, Liang L, Moffatt MF, Chen W, Heath S, Wong KC, Taylor J, Burnett E, Gut I, Farrall M, Lathrop GM, Abecasis GR, Cookson WO. A genome-wide association study of global gene expression. *Nat Genet* 2007: 39(10): 1202-1207.
263. Dudley JT, Tibshirani R, Deshpande T, Butte AJ. Disease signatures are robust across tissues and experiments. *Mol Syst Biol* 2009: 5: 307.
264. Pascual V, Chaussabel D, Banchereau J. A genomic approach to human autoimmune diseases. *Annu Rev Immunol*: 28: 535-571.
265. Mesko B, Poliska S, Nagy L. Gene expression profiles in peripheral blood for the diagnosis of autoimmune diseases. *Trends Mol Med*.
266. Poliska S, Csanky E, Szanto A, Szatmari I, Mesko B, Szeles L, Dezso B, Scholtz B, Podani J, Kilty I, Takacs L, Nagy L. Chronic Obstructive Pulmonary Disease-Specific Gene Expression Signatures of Alveolar Macrophages as well as Peripheral Blood Monocytes Overlap and Correlate with Lung Function. *Respiration*.
267. Wallace AM, He JQ, Burkett KM, Ruan J, Connett JE, Anthonisen NR, Pare PD, Sandford AJ. Contribution of alpha- and beta-defensins to lung function decline and infection in smokers: an association study. *Respir Res* 2006: 7: 76.
268. Oudijk EJ, Nijhuis EH, Zwank MD, van de Graaf EA, Mager HJ, Coffer PJ, Lammers JW, Koenderman L. Systemic inflammation in COPD visualised by gene profiling in peripheral blood neutrophils. *Thorax* 2005: 60(7): 538-544.
269. Chaussabel D, Pascual V, Banchereau J. Assessing the human immune system through blood transcriptomics. *BMC Biol*: 8: 84.
270. Butt C, Gladman D, Rahman P. PPAR-gamma gene polymorphisms and psoriatic arthritis. *J Rheumatol* 2006: 33(8): 1631-1633.
271. El-Sohemy A, Cornelis MC, Park YW, Bae SC. Catalase and PPARgamma2 genotype and risk of rheumatoid arthritis in Koreans. *Rheumatol Int* 2006: 26(5): 388-392.
272. Penyige A, Poliska S, Csanky E, Scholtz B, Dezso B, Schmelczer I, Kilty I, Takacs L, Nagy L. Analyses of association between PPAR gamma and EPHX1 polymorphisms and susceptibility to COPD in a Hungarian cohort, a case-control study. *BMC Med Genet*: 11: 152.
273. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci U S A* 1997: 94(7): 3195-3199.
274. Foreman MG, DeMeo DL, Hersh CP, Carey VJ, Fan VS, Reilly JJ, Shapiro SD, Silverman EK. Polymorphic variation in surfactant protein B is associated with COPD exacerbations. *Eur Respir J* 2008: 32(4): 938-944.

275. Lakhdar R, Denden S, Knani J, Leban N, Daimi H, Hassine M, Lefranc G, Chibani JB, Khelil AH. Microsomal epoxide hydrolase gene polymorphisms and susceptibility to chronic obstructive pulmonary disease in the Tunisian population. *Genet Test Mol Biomarkers*: 14(6): 857-863.
276. Lakatos PL, Altorjay I, Szamosi T, Palatka K, Vitalis Z, Tumpek J, Sipka S, Udvardy M, Dinya T, Lakatos L, Kovacs A, Molnar T, Tulassay Z, Miheller P, Barta Z, Stocker W, Papp J, Veres G, Papp M. Pancreatic autoantibodies are associated with reactivity to microbial antibodies, penetrating disease behavior, perianal disease, and extraintestinal manifestations, but not with NOD2/CARD15 or TLR4 genotype in a Hungarian IBD cohort. *Inflamm Bowel Dis* 2009: 15(3): 365-374.
277. Papp M, Altorjay I, Dotan N, Palatka K, Foldi I, Tumpek J, Sipka S, Udvardy M, Dinya T, Lakatos L, Kovacs A, Molnar T, Tulassay Z, Miheller P, Norman GL, Szamosi T, Papp J, Lakatos PL. New serological markers for inflammatory bowel disease are associated with earlier age at onset, complicated disease behavior, risk for surgery, and NOD2/CARD15 genotype in a Hungarian IBD cohort. *Am J Gastroenterol* 2008: 103(3): 665-681.
278. Papp M, Altorjay I, Norman GL, Shums Z, Palatka K, Vitalis Z, Foldi I, Lakos G, Tumpek J, Udvardy ML, Harsfalvi J, Fischer S, Lakatos L, Kovacs A, Bene L, Molnar T, Tulassay Z, Miheller P, Veres G, Papp J, Lakatos PL. Seroreactivity to microbial components in Crohn's disease is associated with ileal involvement, noninflammatory disease behavior and NOD2/CARD15 genotype, but not with risk for surgery in a Hungarian cohort of IBD patients. *Inflamm Bowel Dis* 2007: 13(8): 984-992.
279. Papp M, Lakatos PL, Harsfalvi J, Farkas G, Palatka K, Udvardy M, Molnar T, Farkas K, Nagy F, Veres G, Lakatos L, Kovacs A, Dinya T, Kocsis AK, Papp J, Altorjay I. Mannose-binding lectin level and deficiency is not associated with inflammatory bowel diseases, disease phenotype, serology profile, and NOD2/CARD15 genotype in a large Hungarian cohort. *Hum Immunol*: 71(4): 407-413.

10 PUBLICATIONS

10.1 Publications related to the thesis

Poliska S, Csanky E, Szanto A, Szatmari I, Mesko B, Szeles L, Dezso B, Scholtz B, Podani J, Kilty I, Takacs L, Nagy L. (2011) Chronic Obstructive Pulmonary Disease-Specific Gene Expression Signatures of Alveolar Macrophages as well as Peripheral Blood Monocytes Overlap and Correlate with Lung Function. **Respiration.** 81:499-510 (2011) IF: 1.935

Penyige A, **Poliska S**, Csanky E, Scholtz B, Dezso B, Schmelczer I, Kilty I, Takacs L, Nagy L. (2010) Analyses of association between PPAR gamma and EPHX1 polymorphisms and susceptibility to COPD in a Hungarian cohort, a case-control study. **BMC Medical Genetics.** 11:152, (2010) IF: 2.84

Poliska S, Penyige A, Lakatos PL and the Hungarian IBD Study Group, Papp M, Palatka K, Lakatos L, Molnar T, Nagy L. Association of peroxisome proliferator-activated receptor gamma polymorphisms to inflammatory bowel diseases in a Hungarian cohort. **Inflammatory Bowel Diseases.** 2011;000:000–000 (DOI 10.1002/ibd.21798 Received for publication May 17, 2011; Accepted May 17, 2011.)

10.2 Other publications

Mesko B, **Poliska S**, Nagy L. (2011) Gene expression profiles in peripheral blood for the diagnosis of autoimmune diseases. Trends Molecular Medicine. 17 (4), 223-233, (2011) IF: 11.049

Horvath, E., Sikovanyecz, J., Pál, A., Kaiser, L., Bálint, B.L., **Póliska, S.,** Kozinszky, Z., Szabó, J., Cystic Dilation of the Aqueductus Sylvii in Case of Trisomy 17p11.2-pter with the Deletion of the Terminal Portion of the Chromosome 6. Case Reports in Medicine. 2010, 1-5, 2010.

Mesko B, **Poliska S**, Szegedi A, Szekanecz Z, Palatka K, Papp M, Nagy L. (2010) Peripheral blood gene expression patterns discriminate among chronic inflammatory diseases and healthy controls and identify novel targets. BMC Medical Genomics. 3 (1) 15, (2010) IF: 2.661

Simandi Z, Balint BL, **Poliska S**, Ruhl R, Nagy L. (2010) Activation of retinoic acid receptor signaling coordinates lineage commitment of spontaneously differentiating mouse embryonic stem cells in embryoid bodies. FEBS Letters. 584(14): 3123-30. (2010) IF: 3.541

Szanto A, Balint BL, Nagy ZS, Barta E, Dezso B, Pap A, Szeles L, **Poliska S**, Oros M, Evans RM, Barak Y, Schwabe J, Nagy L. (2010) STAT6 transcription factor is a facilitator of the nuclear receptor PPARγ-regulated gene expression in macrophages and dendritic cells. Immunity. 33(5): 699-712. (2010) IF: 20.589

Széles L, **Póliska S**, Nagy G, Szatmari I, Szanto A, Pap A, Lindstedt M, Santegoets SJ, Rühl R, Dezsö B, Nagy L. (2010) Research resource: transcriptome profiling of genes regulated by RXR and its permissive and nonpermissive partners in differentiating monocyte-derived dendritic cells. Molecular Endocrinology. (11): 2218-31 (2010) IF: 5.257

Töröcsik D, Baráth M, Benko S, Széles L, Dezso B, **Póliska S**, Hegyi Z, Homolya L, Szatmári I, Lányi A, Nagy L. (2010) Activation of liver X receptor sensitizes human dendritic cells to inflammatory stimuli. Journal of Immunology. 184(10): 5456-65. (2010) IF: 5.646

Széles L, Keresztes G, Töröcsik D, Balajthy Z, Krenács L, **Póliska S**, Steinmeyer A, Zuegel U, Pruenster M, Rot A, Nagy L. (2009) 1,25-dihydroxyvitamin D3 is an

autonomous regulator of the transcriptional changes leading to a tolerogenic dendritic cell phenotype. Journal of Immunology. 182(4): 2074-83. (2009) IF: 5.646

10.3 First author posters on international meetings

Poliska S, Penyige A, Palatka K, Papp M, Nagy L.: Analysis of single nucleotide polymorphisms of PPARγ in chronic inflammatory diseases. EMBO Conference on Nuclear Receptors. Dubrovnik, Croatia. 2009.

Poliska S, Scholtz B, Csanky E, Szatmari I, Szanto A, Nagy L.: Global Gene Expression Analysis of a Complex Human Disease. Functional Genomics & Disease, Innsbruck, Austria, 2008.

Póliska S, Szatmári I, Szántó A, Csánky E, Scholtz B, and Nagy L.: Global Gene Expression Analysis of a Complex Human Disease. EMBO Workshop on Chromatin structure, organization and dynamics, Prague, Czech Repaublic, 2006.

10.4 Presentations

Póliska Szilárd, Csánky Eszter, Scholtz Beáta and Nagy László: A COPD globális génexpressziós analízise. Magyar Tűdőgyógyász Társaság 54. Konferenciája, Szeged, 2006.

11 APPENDIX