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GENETIC FACTORS IN COPD: SPECIAL ATTENTION ON CANDIDATE GENES ENCODING PROTEASES/ANTIPROTEASES AND INFLAMMATORY MEDIATORS

D. Dimov[1], T. Vlaykova[2*]

[1]Dept Internal Medicine, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[2]Dept Chemistry and Biochemistry, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a slowly progressing lung disease characterized by irreversible airflow limitation in the lungs. COPD is a complex disease which is influenced by genetic factors, environmental agents, and gene-environmental interactions. Smoking is one major risk factors for the development of COPD, although other important risk factors have been also considered: occupational exposures, air pollution, age, sex, lung inflammation in childhood, airway hyperreactivity, and genetic predisposition.

Considering the main processes and pathways implicated in pathogenesis of COPD, several genes grouped into 4 main panels, have been proposed and investigated as candidate genes for COPD: i) genes encoding xenobiotic-metabolizing and antioxidant enzymes; ii) genes encoding proteases and antiproteases; iii) inflammatory mediators and iv) genes encoding proteins involved in airway hyperreactivity. In this work we attempted to highlight the recent knowledge and evidence for the role of the candidate genes encoding proteases, antiproteases and inflammatory mediators in genetic susceptibility to COPD. Based on this short review it could be concluded that COPD is a polygenic disease and the individual genetic alterations have a relatively small impact on pathogenesis of this disease. Because the number of the genes and gene polymorphisms proposed to be implicated in the processes of COPD development is growing and the results from the associative and experimental analyses are frequently controversial it is required further substantial exploration.

Key words: COPD, polymorphisms, risk factors, MMPs, ILs

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a slowly progressing lung disease characterized by irreversible airflow limitation in the lungs. COPD is one of the main causes of chronic morbidity and death worldwide. It is considered that until 2020 COPD will be the third leading cause of death after myocardial infarction and insult, and is expected to increase in prevalence until 2030 (1, 2).

Several environmental and endogenous risk factors for COPD have been proposed: they include cigarette smoke, environmental pollution, occupational exposures, age, sex, lung inflammation in childhood, airway hyperreactivity and genetic susceptibility (3-7).

Cigarette smoking is clearly the major environmental determinant of COPD, as the relationship between smoking history and decline in lung function has also been well defined (3-7). However, only 10-20 % of heavy smokers develop severe lung injuries, which are characteristic for COPD. Hence, genetic susceptibility must play a pivotal role in the development of this disease, in addition to environmental factors.

According to the current paradigm for the pathogenesis of chronic obstructive pulmonary disease, the chronic airflow limitation results from an abnormal inflammatory response to inhaled particles and noxious gases in the lung

***Correspondence to:** *Assoc. Prof. Tatyana Vlaykova, PhD, Medical Faculty, Trakia University, Dept Chemistry and Biochemistry, 11 Armeiska Str., Stara Zagora, 6000, Tel: +35942664326, +359898743832 Fax: +35942700702 e-mail: tvlaykov@mf.uni-sz.bg*

leading to enhanced generation of reactive oxygen and/or nitrogen species (ROS/RNS). That ROS/RNS, in addition to those containing in cigarette smoke, result in local oxidative stress which in turn stimulates the immune cells and aggravates the inflammation response. As a consequence of the accelerated inflammation the protease-antiprotease imbalance occurs, which is linked to the pathogenesis of emphysema. Considering the main processes and pathways implicated in pathogenesis of COPD, several genes grouped into 4 main panels **(Figure 1)**, have been proposed and investigated as candidate genes for COPD: i) genes encoding xenobiotic-metabolizing and antioxidant enzymes; ii) genes encoding proteases and antiproteases; iii) inflammatory mediators and iv) genes encoding proteins involved in airway hyperreactivity.

Figure 1. The main processes and pathways involved in pathogenesis of COPD and corresponding groups of candidate genes (According to (8), modified)

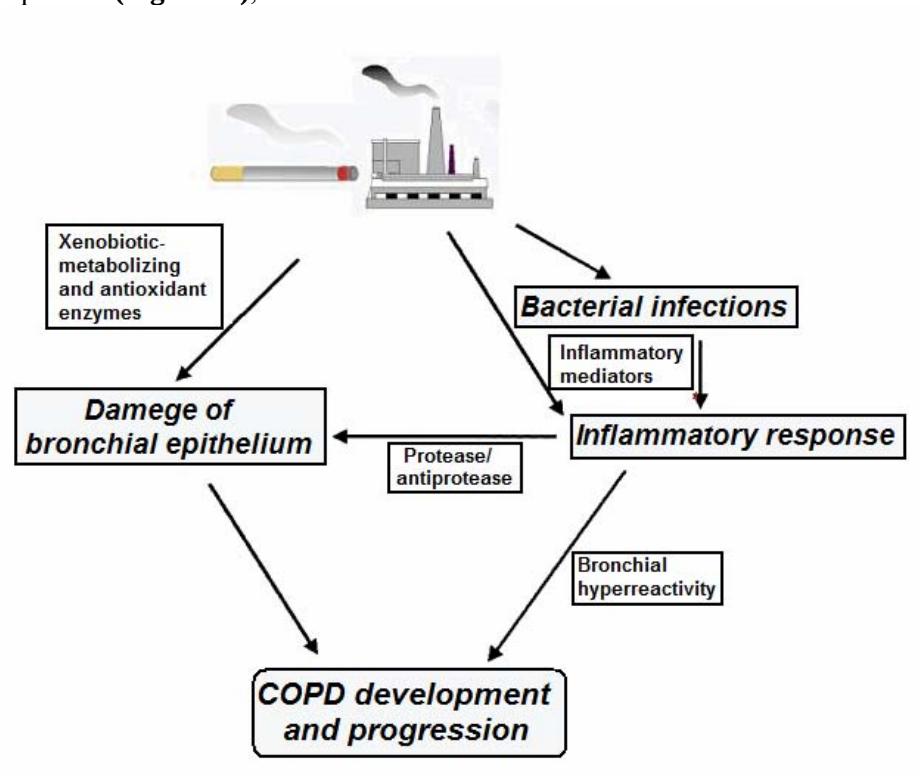

Fig. 1

GENES ENCODING PROTEASES AND ANTIPROTEASES

α1-Antitrypsin (AAT)

Alpha1-antitrypsin (AAT) is the main inhibitor of serum proteases. The inherited deficiency of AAT is the only genetic variation truly proven to be associated with development of COPD **(Table 1)**. AAT belongs to the serpin family, a large group of proteins inhibiting the activity of serine proteases (9). The family consisted also of alpha1-antichymotrypsin, protein C1 inhibitor, antithrombin and neuroserpin. The AAT gene is located on the long arm of chromosome 14 (14q23.1-3) in a cluster with the genes of other serpins. The neutrophil elastase is the main target of AAT: the elastase is attacted and bound by serpin domain, subsequently, inhibited and degraded (9).

The homozygous ZZ patients have a very low level of serum alpha1-antitrypsin and show accelerated rate of decline in lung function (FEV1 % pr.), even in non-smokers (9). The homozygous ZZ smokers develop COPD in early age. However this homozygous genotype is with very low frequency in human population (1/5000) and could explain only about 1% of the cases with COPD.

So far more than 90 different phenotypes of AAT have been determined (10). The most common gene variants are M, S and Z. M1, M2, M3 and M4 are variants of the wild type allele and are detected in about 90% of the populations (9). The Z allele has single nucleotide substitution: G in the M allele is replaced with A in exon 5 in Z allele resulting in substitution of Glu342 with Lis342 in the polypeptide chain of the protein. The altered

protein (with Lis345) is resistant to the enzymatic degradation and could aggregate. The protein accumulates in the endoplasmic reticulum of hepatocytes causing liver damages and diseases and low serum AAT levels. The S allele has a substitution of A (in M allele) with T in exon 3, which results in alteration of Glu264 with Val264 in polypeptide chain of the inhibitor.

It has been shown that some genotypes (ZZ, SZ, MZ, SS, MS) are associated with decrease of serum concentration of α1-AT about 16%, 51%, 83%, 93% and 97%, respectively in comparison to the wild-type MM genotype. The homozygous ZZ genotype results in the most severe тежък α1-AT deficiency (9, 10) **(Table 1)**.

Some investigations have compared individuals with MZ genotype with those with MM genotype and have reported significant differences of lung functions or symptoms among nonsmokers. However there are controversial results among smokers. It has been shown that MZ smokers have greater loss of elastic recoil than MM smokers and more rapid decline in FEV1 (9-11).

The SS and SM genotypes do not contribute significantly to development of COPD (9). The heterozygous SZ genotype has been associated with less severe airway limitations than the ZZ genotype. The SZ smokers have been reported to have decline of lung function and 5-fold higher risk of airway obstruction compared to smokers with MM genotype (12) **(Table 1)**.

α1-Antichymotrypsin (a1-ACT)
The alpha1-antichymotrypsin (a1-ACT) is a protease inhibitor secreted by hepatocytes and alveolar macrophages. This gene has several polymorphisms. The variant Ala allele of Thr-15Ala SNP of *a1-ACT resulting in* a1-ACT deficiency has been shown to associate with COPD in a Swedish population (13), however it has no effect on the risk for COPD in Italian population (14) **(Table 1)**.

α2-Macroglobulin (A2M)
The gene of α2-macroglobulin is localized on chromosome 12. A2M is inhibitor with a wide range antiprotease activity. It is synthesized in hepatocytes and alveolar macrophages. The serum deficiency is a rare event. The most common variation of A2M gene is 5'-nucleotide deletion. The inherited deficiency of A2M, although rare, has been associated with 20-30-fold higher risk of COPD (10, 15) **(Table 2)**.

Secretory leukocyte proteinase inhibitor (SLPI)
The secretory leukocyte proteinase inhibitor (SLPI) is thought to be one of the most powerful antiproteinase inhibitors in the airways, which is produced by bronchial epithelial cells. It is very active against the neutrophil elastase. In addition this important antiproteinase inhibitor possesses antibacterial and antiviral activity. In general the gene of SLPI is relatively stable, non-polymorphic, and it is constitutively expressed in different tissues. However it has been suggested that the gene expression could be modulated on transcriptional and posttranscriptional levels (16). Recently, a study in UK (17) has shown that in sputum of COPD patients with frequent exacerbations the levels of SLP are low, which suggests the role of this inhibitor in progression of the disease.

Matrix matalloproteinases (MMPs)
He matrix metalloproteinases (MMP's) belong to a large family consisted of more than 30 proteolytic enzymes (18, 19), which play a pivotal role in tissue remodeling and repair in development and inflammatory processes (20).

Over-expression of MMPs is associated with severe pathological conditions, such as irreversible degradation of tissues in arthritis, degradation of collagen in tumor invation and matastasing leading to unfavorable prognosis of the patients (18, 21).

Studies with animals and huan have shown that MMP-1 (interstitial collagenase), MMP-12 (human macrophage elastase) and MMP-9 (gelatinase B) are important in inflammatory processes in airways of lungs and for development of emphysema (20, 22). Transgenic animals over-expressing human MMP-1 in the lungs developed morphological changes resembling emphysema in human (23). On the other hand, MM-12 knockout mice did not develop emphysema after cigarette smoke exposure in comparison to the wild type mice (24). This observation has suggested the critical role of MMP-12 for development of lung injuries induced by cigarette smoke. Moreover, smokers with airway obstruction had over-expression of MMp-1 and MMP-9 in comparison to smokers without COPD and nonsmokers (25).

Table 1. *Candidate genes encoding proteases and antiproteases, the corresponding candidate risk alleles, their effects and results from investigations for elucidation their role for development of COPD.*

Candidate gene	*Candidate risk allele*	*Effect of the risk allele*	*Results from associative studies*
α1-Antitrypsin (AAT)	**Z** (Lys342)(9, 10)	α1-AT deficiency (15-16% of normal values)	*ZZ* genotype- high risk of COPD *MZ* – moderate risk (9-11)
	S (Val 264) (9, 10)	α1-AT deficiency (60% of normal values)	No effect on the risk
a1-Antichymotrypsin (AACT)	Ala 227	α1-ACT deficiency (51% of normal values)	Association with COPD in some populations(13, 14)
	Thr-15	Unknown	Association with COPD(13, 14)
α2-Macroglobulin (A2M)	Tyr 792	Affects protein function	Association with COPD in one study(10, 13)
MMP1	-1607GG	Creates a new binding site for ETS-1 transcription factor	Decline of lung function(31)
MMP9	-1562T	Lower affinity of promoter to the transcriptional repressor	3-fold increased risk for emphysema(32)
MMP12	-82G	Decrease affinity of the promoter to the transcriptional factor AP-1	Positively associated with FEV1 and with reduced risk of COPD(30)
	Asn357	Unknown	*MMP12 Asn357* in combination with *MMP1 -1607GG* – decline of lung function(31)
TIMP2	853G	Decreases transcription and mRNA stability	Higher proportion in COPD patients (10, 34)
	-418C		
ADAM33 (a disintegrin and metalloprotease 33)	T2G (39)	Affects signaling, being located within the putative SH3-binding domain	Association with COPD (39, 47)
	T1G (39)	Unknown	
	S2C(39), S2G(47)	Affects signaling, being located within the putative SH3-binding domain	
	V4C(47)	Unknown	
	Q-1G(39), Q-1C(47)	Located near the EGF domain and can modulate lung morphogenesis	Association with COPD and lower lung function (47)
	S1G(47)	Unknown	
	V-1C(47)	Unknown	

Large number of promoter polymorphisms has been determined in the genes of MMPs resulting in alteration of the gene expression (26-29). The promoter polymorphism of MMP1 gene (G-1607GG) introduces a new binding site for the transcriptional factor ETS-1 (26). The substitution of A with G at position -82 (A-82G) in *MMP12* results to decrease affinity of the promoter to the transcriptional factor AP-1 (27), which in turn leads to decrease expression of MMP-12. The minor allele (G) of this functional variant in the promoter region of MMP12 (A−82G) was positively associated with FEV1 in a combined analysis of children with asthma and adult former and current smokers in all cohorts.
This allele was also associated with a reduced risk of COPD (30).

The investigations of the role of other promotor polymorphisms in lung diseases have shown that the variant -1607GG allele of the G-1607GG polymorphism of MMP1 is associated with decline of lung function of smokers (31). In the same study, it was reported that polymorphisms in MMP-9 and MMP-12 did not have individual effect, however the combination (the haplotype) -1607GG/MMP1 and Asn375/MMM12 showed statistically significant association with the decline of lung function (31). Alternatively, in another study it as been shown that the promoter polymorphism of *MMP9* gene (C-1562T) was associated with development of emphysema in Japanese smokers (32): the variant -1562T allele correlated with about 3-fold increased risk for emphysema (32). This risk allele had lower affinity to the transcriptional repressor (33).

Tissue inhibitors of MMP (TIMPs)
The functions of MMPs are controlled by their tissue inhibitors (TIMPs). In the gene encoding the inhibitor f MMP-2 (TIMP-2) two polymorphisms (G-418C and G853A) have been studied in patients with COPD. The allele frequencies of 853G and -418C have been reported to be significantly higher in patients in comparison to the controls. These authors suggested that the studied polymorphisms were associated with COPD because they resulted in decreased transcriptional activity and destabilization of mRNA (10, 34).

A disintegrin and metalloprotease 33 (ADAM33)
ADAM33 belongs to the ADAM (A Disintegrin And Metalloproteass) family of membrane anchored metalloproteinases, capable of shedding a multitude of proteins from the surface of the cell (35, 36). Mammalian ADAMs are involved in various biological and disease-related processes, such as cell-cell fusion, adhesion and intracellular signaling. Functional activity of ADAMs have been described in sperm-egg binding and fusion, trophoblast invasion and matrix degradation during pregnancy, angiogenesis and neovascularization. ADAMs are implicated in pathological processes, including cancer, inflammation, neurodegeneration, asthma and fibrosis, through shedding of the apoptosis-inducing FAS ligand, cytokines and growth factors (36, 37).

A second group of proteins within the ADAM family has recently been discovered: ADAMTS (ADAM with thrombospondin domains) family. These proteins contain several thrombospondin-like repeats in their C-terminal regions and lack the transmembrane domain known to be present in ADAMs (36, 37).

The data concerning the expression of members of ADAM and ADAMTS families in lung tissues are more recent and rather incomplete (37). In lung tissue, an expression of ADAM-8, -9, -10, -12, -15, -17 and ADAMTS-1, -2 has been observed (37, 38).

Over the last years, the attention has risen about the roles of ADAM proteinases in processes leading to airway diseases such as asthma and COPD. ADAM-33 was one of the first ADAM proteinases to be identified as an asthma susceptibility gene (37).

ADAM33 is an active proteinase that is able to cleave α2-macroglobulin and synthetic peptides (39). The enzymatic activity of ADAM33 can be inhibited by tissue inhibitor of metalloproteinase- 3 and -4 (TIMP-3 and -4, respectively) as well as several small molecules (39, 40). It has been showed that a truncated, soluble form of ADAM33 containing the catalytic domain caused rapid induction of endothelial cell differentiation in vitro and angiogenesis ex vivo and in vivo. Although not as well studied, there is evidence that the vascular area of the airway is significantly increased in COPD and that this increase correlates with the degree of airflow obstruction (40). In addition there are studies describing ADAM33 gene expression in airway smooth muscle cells and fibroblasts in the lung (39, 40), suggesting that it is important in the development of asthma and in other lung disease, possibly through airway remodeling (41).

Genome-wide screening has revealed that chromosome 20p13 was significantly linked to asthma and airway hyperresponsiveness. This genomic region contains the gene ADAM33 (42). Since the first report of an association between ADAM33 polymorphisms and asthma, many studies have confirmed this relation of ADAM-33 gene polymorphisms with the hyperresponsiveness and asthma (41, 43-46).

Lately, ADAM-33 has also been identified as a susceptibility gene for COPD since single nucleotide polymorphisms (SNPs) observed in this gene wereassociated with a higher risk for developing COPD (39, 46, 47). ADAM-33 has recently been reported to be linked to airway hyperresponsiveness and airway inflammation in the general population suffering from COPD (35, 47).

Several polymorphisms in the promoter and other regulatory regions as well as in coding regions of *ADAM33* gene have been studied in association with COPD.

The -2154G/A SNP in the promoter region was assumed to alter the gene's transcriptional rate, the F+1 SNP (7575 G/A in intron 6) is close to Cys179 (cysteine switch), potentially modulating proteolytic activity, the L_1 ($Ala^{359}Val$) is located in the catalytic section of ADAM33, whereas the T2 (12462 G/A, $Pro^{774}Ser$) and S2 10918 (G/C, $Gly^{717}Gly$)

SNPs may also affect signalling, being located within the putative SH3-binding domain. The Q-1 (G/A) SNP is located near the EGF domain and can modulate lung morphogenesis.

The analyses of 8 SNPs in ADAM33 [F+1 (G/A in intron 6), Q-1 (intronic C/T), S1 (A/G in exon 19, $Val^{710}Ile$), S2 (exonic C/G, $Gly^{717}Gly$), ST+5 (intronic A/G), T1 (A/G, $Met^{764}Thr$), T2 (A/G, $Pro^{774}Ser$), V4 (exonic C/G)], Wang et al. have found statistically significant differences in the distributions of the T2G, T1G, S2C, and Q-1G alleles between patients and controls, and showed that all of these risk alleles determined about 2-3-fold higher risk for COPD (39). In another study, however Q-1C, S2G, S1G, V4C and V-1C of the corresponding Q-1 (C/T), S2 (G/C), S1 (G/A), V4 (C/G) and V-1 (C/A) SNPs were statistically associated with COPD) (47). In addition, Q-1C, S1G and V-1C were also associated with lower ppFEV1, FEV1/FVC ratio and ppFEF25–75. (47)

Table 2. *Candidate genes encoding inflammatory mediators, the corresponding candidate risk alleles, their effects and results from investigations for elucidation their role for development of COPD.*

Candidate gene	*Candidate risk allele*	*Effect of the risk allele*	*Results from associative studies*
Vitamine-D binding protein (VDBP)	Gc1F	Possibly affect the conversion to macrophage-activating factor	Increased risk of COPD, conflicting results.(10, 49)
	Gc2		Decreased risk of COPD(50)
*Tumor necrosis factors-*α (TNF-α)	-308A	Enhanced expression of TNF-α and increased serum levels	Increased risk of COPD, conflicting results.(10, 51, 52)
Interleukine-11 (IL11)	Dinucleotide microsatellite promoter allele, *IL11A2*	Alteration of the expression	Protective effect for COPD(53)
IL1 *(IL1B)*	-511T	Increased levels of IL-1 and ILRN	In combination with Allele2 of *ILRN* – risk factor of fasr decline of lung function (55)
IL-1 receptor antagonist (ILRN, ILRa)	Allele 2	Unknown	Association with COPD(55)
IL13	-1055T	Altered regulation of expression, increased levels	Risk factor of COPD(56)
	1103C	Lower level of IL-13	Decreased risk of COPD(57)
*Transforming growth factor-*β*l* (*TGFB1)*	-509T	Increased affinity to YY1 nuclear factor and increased expression	Association with COPD(61)
	29C	Increased production of TGF-β	
IL10	-1082A	Reduced IL-10 production	Risk factor of COPD(79) and decline of lung function (71). Controversial results (76, 77)

GENES ENCODING INFLAMMATORY MEDIATORS

Vitamin-D-binding protein (VDBP)

Vitamin-D-binding protein (VDBP) (Gc globulin) is a protein secreted by liver. It binds vitamin D, can act as macrophage activating factor and can increase the chemoatracting activity of C5a for neutrophils (48). Thus, VDBP is capable to regulate the inflammatory response or to decline the antioxidant capacity of patients. Functionally active VDBP is found in broncho-alveolar lavage, as higher levels of this protein are assessed in patients with COPD and in asymptotic smokers compared to non-smokers (10).

There are three main isotypes of this serum proteins encoded by three co-dominant alleles of Gc-globuline gene: Gc1S, Gc1F and Gc2 (49). These three alleles are found with the following frequency in the Caucasian populations: 0.56, 0.16 and 0.28 (50). The differences between the three isoforms are results from two SNPs each of which leads to a amino acid substitution. There are studies on the associations of the three most common

polymorphisms in VDBP gene with COPD, however the results are conflicting. In a study, it has been found that Gc2 allele is protective for COPD (50), whereas in other investigations the homozygous Gc1F individuals had increased risk for COPD (10, 49). It has been found that COPD patients with Gc1F allele have faster decline in FEV1 (49).

Tumor necrosis factors (TNFs)

Tumor necrosis factor-α (TNF-α) and TNF-β are pro-inflammatory cytokines with multiple effects that might be implicated in pathogenesis of COPD: activating of neutrophils and releasing by them of different mediators and proteinase (elastase). TNF-α s supposed to play an important role in induction of apoptosis. Alveolar septa cell apoptosis is assumed to contribute to development of emphysema. The -308G>A promotor polymorphism in TNF-α gene has been proven to functional: the allele 2 (-308A) was shown to be associated with altered (increased) secretion of the cytokine in vitro. The studies on the role of polymorphisms in promoter region of TNF-α gene for development of COPD have reported quite controversial results (51, 52, Tzortzaki, 2006 #173). Another 4 SNPs in regulatory regions of TNF-α gene have been analyzed in association with COPD: a promoter SNP (-238), a SNP in intron (+489), and 2 SNPs in 3′ UTR. The results were negative (52).

Interleukin-11 (IL11)

IL-11 is a cytokine with an anti-inflammatory effect expressed mainly by reduction of the levels of TNF-α and sequestration of neutrophils that has been demonstrated in in vivo studies (53). The investigations on the role of IL-11 in genetic susceptibility to COPD have been focused on a dinucleotide microsatellite polymorphism in promotor of the gene. It has been found that *IL11A2* microsatellite allele and *IL11A2* homozygous genotype are significantly less frequent in COPD patients than in controls. This difference in the frequencies is even more pronounced in patients nonsmokers compared to controls nonsmokers (53). These result suggested about the protective role of *IL11A2* microsatellite allele for development of COPD.

Interleukine-1 family (*IL1*)

Currently the family of IL-1 consisted of 11 members with opposed effects on the inflammatory processes (54). Among them attention has been paid on the two main pro-inflammatory cytokines IL-1α and IL-1β and on one of the natural anti-inflammatory agents, IL-1 receptor antagonist (ILRN, ILRa) (54, 55). The two forms of IL-1 are coded by two diverse genes, however they have significant structural homology and bind to the same receptor. They are synthesized by variety of cell types, including monocytes and macrophages. IL-1 and its antagonist can influence the lung function due to the effect of IL-1 on the neutrophil functions and chemotaxis and induction of neutrophil elastase release. IL-1 assists the adhesion of the neutrophils via stimulation of adhesion molecule expression such as ICAM-1, VCAM-1 and L-Selectin (54).

The genes of IL-1α, IL-1β and ILRN are localized on the long arm of chromosome 2 and each of them is polymorphic. The gene of IL-1β *(IL1B)* has a SNPin the promoter region (C–511T), whereas *IL1RN* gene possesses a penta-alellic polymorphic site in intron 2 containing 2-6 tandem repeats of 86 bp. Evidence has been accumulated that allele 2 of *IL1RN (IL1RN*2)* has been associated with susceptibility to and worse outcome from several chronic inflammatory diseases (ulcerative colitis and systemic lupus erythematosus) (54, 55). The promoter polymorphism in IL1B (C-511T) has also been linked with inflammatory bowel diseases and plasma levels of IL-1β and IL-1RN (55).

Both polymorphisms have no independent effect on the risk for COPD, however the *IL1B/IL1RN* haplotype has appeared to be important for decline of lung function in smokers : allele 1 of *IL1RN* gene in combination with -511T allele of *IL1B* has turned out to be risk factors for rapid decline of lung function (55).

Interleukine-13 (IL13)

The gene of human IL-13 together with that of IL-4 are localized on the long arm of chromosome 5 (5q31), a region that has been lined with airway hyper-responsiveness, asthma and serum levels of IgE.

It has been found that the inhibition of IL-13 activity in the lung of sensitized mice prevents several characteristics of asthma, such as airway hyper-responsiveness, pulmonary eosinophilia and mucus production, whereas the lung expression of transgenic IL-13 results

in a COPD phenotype with inflammation, mucus metaplasia and matrix-metalloproteinase- and cathepsin-dependent emphysema (56). These results have unequivocally indicated the unique role of IL-13 in pathogenesis of COPD (56). Van der Pouw Kraan et al. have also reported an increased frequency of the -1055T allele of C-1055T SNP of *IL13* gene in COPD patients compared to healthy controls and to smoking individuals with normal lung function (56).

In another study 3 SNPs in IL13 gene have been investigated as risk factors of COPD: 1103C/T, 4257G/A, 4738G/A. The results have indicated that CC genotype and C allele of 1103C/T SNP is more common in controls than in COPD patients and are associated with lower levels of IL-13 suggesting that these genotype and allele are protective for COPD (57).

Transforming growth factor-β1 (TGFB1)
In vitro, TGF-ß1 regulates the immune response, growth and differentiation of the cells, tissue reparation and production of exstracellular matrix. Investigations with animal models have provided strong evidence that changes in activation and signalization of TGF-ß1 are implicated in pathogenesis of lung emphysema. Mice lacking integrin-mediated activation of the latent TGF-ß1 (Itgb6-null) have developed lung emphysema accompanied with altered expression of MMP-12 by macrophages (58). These results clearly indicated the role of TGF-β1 in pathogenesis f COPD.

In addition it has been found that the long arm of chromosome 19 contains genetic locus/loci which are shown to influence the development of COPD (59). In this region of chromosome 19 the gene of TGF-β is also located suggesting it as a candidate gene for COPD. Several polymorphisms in the gene of TGF-β1 have been determined, as those with a functional effect are the following: C-509T SNP in the promoter region and T29C SNP in exon 1 of the gene. The T allele of C-509T SNP in the promoter region of TGF-β1 alters the binding site for Ying Yang 1 (YY1) transcriptional factor resulting in enhancement of the affinity for the factor and activity of the promoter. As a consequence the serum level of the cytokine TGF-ß1 is increased. The variant C allele of T29C SNP in exon 1 of *TGFB1* gene, which leads to replasment of Leu with Pro at codon 10 (Leu10Pro) has also been shown to increase the production of TGF-ß1 (60).

In a large investigation for elucidation of the role of polymorphisms in *TGFB1* gene, it has been found that both SNPs, C-509T and T29C, in TGFB1 are associated with COPD (61).

Interleukine 10 (IL10)
IL-10 is an important immunoregulatory cytokine with pleiotropic effects (62, 63). IL-10 has a key role in controlling the balance between humoral and cell (Th1/Th2) immune response. IL-10 possesses strong immunosuppresor effects carried out both via stimulation of proliferation and differentiation of B and Th2 cells and via inhibition of pro-inflammatory Th1 lymphocytes and pro-inflammatory activity of macrophages. As a consequence of the macrophage inhibition there is decreased expression and release of TNF-α and IL-8 by macrophages (64). Information about the expression and involvement of IL-10 in regulation of inflammatory processes in COPD are quite limited (65). It has been found that IL-10 concentration in induced sputum of COPD patients and smokers was lower than non-smokers (65), while there were increased levels of TNF-α and IL-8 and impaired protease/antiprotease balance shifted into the proteases (66).

Based on that experimental evidence, it has been proposed a hypothesis about the role of decreased production of inhibitory cytokines, including IL-10, in development of chronic inflammation in airways assigned to Bronchial asthma and COPD. (65).

The gene of IL-10 *(IL10)* is localized on the long arm of chromosome 1 (1q31-32). The gene of human IL-10 is highly polymorphic: more than 51 polymorphisms have been detected until now. Many of those polymorphisms (28) are located in the promoter region of the gene, most of which affect the expression of the cytokine. According to the research literature the most important functional polymorphism is the substitution of A>G at position -1082 ($A^{-1082}G$; -1082A/G; rs 1800896) (67-71). This SNP is localized in a binding site for a transcriptional factor resulting in altered production and secretion of IL-10 (72). The A lellele at position -1082 in *IL10* gene has been shown to determine lower production of the cytokine,

while the variant G allele on opposite – it s associated with higher production of IL-10 (68, 70, 73). The same correlation between this polymorphism and IL-10 serum levels have been reported for COPD patients with severe α1-antitrypsin deficiency (71). That polymorphism, as well as other ones have been found to be associated with FEV1 of patients with asthma (74) and with the rate of decline of lung function in firefighters (75). There are only few reports about the role of -1082A/G SNP in IL10 in COPD development and the results are conflicting (69, 71, 76-78).

Our own preliminary results concerning genotypes and allele distributions of -1082A/G SNP of *IL10* in 118 patients with COPD (stages II, III and IV) and 116 healthy controls indicated that the frequency of the A allele and homozygous AA genotype was significantly more common in controls than in patients and was associated, although not significantly, with lower characteristics of lung function (79) . Thus we suggested that -1082A/G SNP of IL10 might be involved in genetic susceptibility to COPD as the low-producing A allele is a risk allele possibly via shifting the anti-/pro-inflammatory cytokine balance to the latter once in airways resulting in augmentation of inflammatory process (79).

CONCLUSION

Although there is clear evidence of a genetic contribution to the pathogenesis of COPD, only few specific genes have been implicated. Those specific candidate genes encode proteins involved in different processes and pathways composing the pathogenetic mechanisms of development and progression of COPD: i) xenobiotic-metabolizing and antioxidant enzymes; ii) proteases and antiproteases; iii) inflammatory mediators and iv) proteins involved in airway hyperreactivity. It is apparent that the number of the genes and gene variants proposed to be implicated in the processes of COPD development is growing, which requires further substantial exploration. Based on the short review we have attempted to perform it could be concluded that the number of the genes and gene polymorphisms proposed to be implicated in the processes of COPD development is growing and the results from the associative and experimental analyses are frequently controversial, which requires further substantial exploration.

REFERENCES

1. Murtagh, E., Heaney, L., Gingles, J., Shepherd, R., Kee, F., Patterson, C. and MacMahon, J., Prevalence of obstructive lung disease in a general population sample: the NICECOPD study. *Eur J Epidemiol*, 20:443-453, 2005.
2. MacNee, W., Update in chronic obstructive pulmonary disease 2007. *Am J Respir Crit Care Med*, 177:820-829, 2008.
3. Doll, R., Peto, R., Boreham, J. and Sutherland, I., Mortality in relation to smoking: 50 years' observations on male British doctors. *Bmj*, 328:22, 2004.
4. Mannino, D.M. and Braman, S., The epidemiology and economics of chronic obstructive pulmonary disease. *Proc Am Thorac Soc*, 4:502-506, 2007.
5. Teramoto, S., 1. COPD pathogenesis from the viewpoint of risk factors. *Intern Med*, 46:77-79, 2007.
6. MacNee, W., Oxidants and COPD. *Curr Drug Targets Inflamm Allergy*, 4:627-641, 2005.
7. MacNee, W., Pathogenesis of chronic obstructive pulmonary disease. *Proc Am Thorac Soc*, 2:258-266, 2005.
8. Joos, L., Pare, P.D. and Sandford, A.J., Genetic risk factors of chronic obstructive pulmonary disease. *Swiss Med Wkly*, 132:27-37, 2002.
9. Ekeowa, U.I., Gooptu, B., Belorgey, D., Hagglof, P., Karlsson-Li, S., Miranda, E., Perez, J., MacLeod, I., Kroger, H., Marciniak, S.J., Crowther, D.C. and Lomas, D.A., alpha1-Antitrypsin deficiency, chronic obstructive pulmonary disease and the serpinopathies. *Clin Sci*, 116:837-850, 2009.
10. Tzortzaki, E.G., Tsoumakidou, M., Makris, D. and Siafakas, N.M., Laboratory markers for COPD in "susceptible" smokers. *Clinica Chimica Acta*, 364:124-138, 2006.
11. Dahl, M., Tybjaerg-Hansen, A., Lange, P., Vestbo, J. and Nordestgaard, B.G., Change in lung function and morbidity from chronic obstructive pulmonary disease in alpha1-antitrypsin MZ heterozygotes: A longitudinal study of the general population. *Ann Intern Med*, 136:270-279, 2002.
12. Dahl, M., Nordestgaard, B.G., Lange, P., Vestbo, J. and Tybjaerg-Hansen, A., Molecular diagnosis of intermediate and severe alpha(1)-antitrypsin deficiency: MZ individuals with chronic obstructive

pulmonary disease may have lower lung function than MM individuals. *Clin Chem*, 47:56-62, 2001.
13. Poller, W., Faber, J.P., Scholz, S., Weidinger, S., Bartholome, K., Olek, K. and Eriksson, S., Mis-sense mutation of alpha 1-antichymotrypsin gene associated with chronic lung disease. *Lancet*, 339, 1992.
14. Benetazzo, M.G., Gile, L.S., Bombieri, C., Malerba, G., Massobrio, M., Pignatti, P.F. and Luisetti, M., alpha 1-antitrypsin TAQ I polymorphism and alpha 1-antichymotrypsin mutations in patients with obstructive pulmonary disease. *Respir Med*, 93:648-654, 1999.
15. Poller, W., Barth, J. and Voss, B., Detection of an alteration of the alpha 2-macroglobulin gene in a patient with chronic lung disease and serum alpha 2-macroglobulin deficiency. *Hum Genet*, 83:93-96, 1989.
16. Abe, T., Kobayashi, N., Yoshimura, K., Trapnell, B.C., Kim, H., Hubbard, R.C., Brewer, M.T., Thompson, R.C. and Crystal, R.G., Expression of the secretory leukoprotease inhibitor gene in epithelial cells. *J Clin Invest*, 87:2207-2215, 1991.
17. Gompertz, S., Bayley, D.L., Hill, S.L. and Stockley, R.A., Relationship between airway inflammation and the frequency of exacerbations in patients with smoking related COPD. *Thorax*, 56:36-41, 2001.
18. Back, M., Ketelhuth, D.F. and Agewall, S., Matrix metalloproteinases in atherothrombosis. *Prog Cardiovasc Dis*, 52:410-428, 2010.
19. Louhelainen, N., Stark, H., Mazur, W., Rytila, P., Djukanovic, R. and Kinnula, V.L., Elevation of sputum matrix metalloproteinase-9 persists up to 6 months after smoking cessation: a research study. *BMC Pulm Med*, 10:13, 2010.
20. Crosby, L.M. and Waters, C.M., Epithelial Repair Mechanisms in the Lung. *Am J Physiol Lung Cell Mol Physiol*, 298:L715-731, 2010.
21. Nikkola, J., Vihinen, P., Vlaykova, T., Hahka-Kemppinen, M., Kahari, V.M. and Pyrhonen, S., High expression levels of collagenase-1 and stromelysin-1 correlate with shorter disease-free survival in human metastatic melanoma. *Int J Cancer*, 97:432-438, 2002.
22. Lagente, V. and Boichot, E., Role of matrix metalloproteinases in the inflammatory process of respiratory diseases. *J Mol Cell Cardiol*, 48:440-444, 2009.
23. D'Armiento, J., Dalal, S.S., Okada, Y., Berg, R.A. and Chada, K., Collagenase expression in the lungs of transgenic mice causes pulmonary emphysema. *Cell*, 71:955-961, 1992.
24. Hautamaki, R.D., Kobayashi, D.K., Senior, R.M. and Shapiro, S.D., Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science*, 277:2002-2004, 1997.
25. Segura-Valdez, L., Pardo, A., Gaxiola, M., Uhal, B.D., Becerril, C. and Selman, M., Upregulation of gelatinases A and B, collagenases 1 and 2, and increased parenchymal cell death in COPD. *Chest*, 117:684-694, 2000.
26. Rutter, J.L., Mitchell, T.I., Buttice, G., Meyers, J., Gusella, J.F., Ozelius, L.J. and Brinckerhoff, C.E., A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res*, 58:5321-5325, 1998.
27. Jormsjo, S., Ye, S., Moritz, J., Walter, D.H., Dimmeler, S., Zeiher, A.M., Henney, A., Hamsten, A. and Eriksson, P., Allele-specific regulation of matrix metalloproteinase-12 gene activity is associated with coronary artery luminal dimensions in diabetic patients with manifest coronary artery disease. *Circ Res*, 86:998-1003, 2000.
28. Zhang, B., Ye, S., Herrmann, S.M., Eriksson, P., de Maat, M., Evans, A., Arveiler, D., Luc, G., Cambien, F., Hamsten, A., Watkins, H. and Henney, A.M., Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation*, 99:1788-1794, 1999.
29. Shimajiri, S., Arima, N., Tanimoto, A., Murata, Y., Hamada, T., Wang, K.Y. and Sasaguri, Y., Shortened microsatellite d(CA)21 sequence down-regulates promoter activity of matrix metalloproteinase 9 gene. *FEBS Lett*, 455:70-74, 1999.
30. Hunninghake, G.M., Cho, M.H., Tesfaigzi, Y., Soto-Quiros, M.E., Avila, L., Lasky-Su, J., Stidley, C., Melen, E., Soderhall, C., Hallberg, J., Kull, I., Kere, J., Svartengren, M., Pershagen, G., Wickman, M., Lange, C., Demeo, D.L., Hersh, C.P.,

Klanderman, B.J., Raby, B.A., Sparrow, D., Shapiro, S.D., Silverman, E.K., Litonjua, A.A., Weiss, S.T. and Celedon, J.C., MMP12, lung function, and COPD in high-risk populations. *N Engl J Med*, 361:2599-2608, 2009.

31. Joos, L., He, J.Q., Shepherdson, M.B., Connett, J.E., Anthonisen, N.R., Pare, P.D. and Sandford, A.J., The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. *Hum Mol Genet*, 11:569-576, 2002.
32. Minematsu, N., Nakamura, H., Tateno, H., Nakajima, T. and Yamaguchi, K., Genetic polymorphism in matrix metalloproteinase-9 and pulmonary emphysema. *Biochem Biophys Res Commun*, 289:116-119, 2001.
33. Ugenskiene, R., Sanak, M., Sakalauskas, R. and Szczeklik, A., Genetic polymorphisms in chronic obstructive pulmonary disease. *Medicina*, 41:17-22, 2005.
34. Sandford, A.J. and Parй, P.D., Genetic Risk Factors for Chronic Obstructive Pulmonary Disease. *Clinics in Chest Medicine*, 21:633-643, 2000.
35. Gosman, M.M., Boezen, H.M., van Diemen, C.C., Snoeck-Stroband, J.B., Lapperre, T.S., Hiemstra, P.S., Ten Hacken, N.H., Stolk, J. and Postma, D.S., A disintegrin and metalloprotease 33 and chronic obstructive pulmonary disease pathophysiology. *Thorax*, 62:242-247, 2007.
36. van Goor, H., Melenhorst, W.B., Turner, A.J. and Holgate, S.T., Adamalysins in biology and disease. *J Pathol*, 219:277-286, 2009.
37. Paulissen, G., Rocks, N., Gueders, M.M., Crahay, C., Quesada-Calvo, F., Bekaert, S., Hacha, J., El Hour, M., Foidart, J.M., Noel, A. and Cataldo, D.D., Role of ADAM and ADAMTS metalloproteinases in airway diseases. *Respir Res*, 10:127, 2009.
38. Rocks, N., Paulissen, G., Quesada Calvo, F., Polette, M., Gueders, M., Munaut, C., Foidart, J.M., Noel, A., Birembaut, P. and Cataldo, D., Expression of a disintegrin and metalloprotease (ADAM and ADAMTS) enzymes in human non-small-cell lung carcinomas (NSCLC). *Br J Cancer*, 94:724-730, 2006.
39. Wang, X., Li, L., Xiao, J., Jin, C., Huang, K., Kang, X., Wu, X. and Lv, F., Association of ADAM33 gene polymorphisms with COPD in a northeastern Chinese population. *BMC Med Genet*, 10:132, 2009.
40. Brew, K. and Nagase, H., The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. *Biochim Biophys Acta1803*, 2010:55-71, 2010.
41. Holgate, S.T., Davies, D.E., Powell, R.M. and Holloway, J.W., ADAM33: a newly identified protease involved in airway remodelling. *Pulm Pharmacol Ther*, 19:3-11, 2006.
42. Van Eerdewegh, P., Little, R.D., Dupuis, J., Del Mastro, R.G., Falls, K., Simon, J., Torrey, D., Pandit, S., McKenny, J., Braunschweiger, K., Walsh, A., Liu, Z., Hayward, B., Folz, C., Manning, S.P., Bawa, A., Saracino, L., Thackston, M., Benchekroun, Y., Capparell, N., Wang, M., Adair, R., Feng, Y., Dubois, J., FitzGerald, M.G., Huang, H., Gibson, R., Allen, K.M., Pedan, A., Danzig, M.R., Umland, S.P., Egan, R.W., Cuss, F.M., Rorke, S., Clough, J.B., Holloway, J.W., Holgate, S.T. and Keith, T.P., Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature*, 418:426-430, 2002.
43. Werner, M., Herbon, N., Gohlke, H., Altmuller, J., Knapp, M., Heinrich, J. and Wjst, M., Asthma is associated with single-nucleotide polymorphisms in ADAM33. *Clin Exp Allergy*, 34:26-31, 2004.
44. Blakey, J., Halapi, E., Bjornsdottir, U.S., Wheatley, A., Kristinsson, S., Upmanyu, R., Stefansson, K., Hakonarson, H. and Hall, I.P., Contribution of ADAM33 polymorphisms to the population risk of asthma. *Thorax*, 60:274-276, 2005.
45. Holgate, S.T., Yang, Y., Haitchi, H.M., Powell, R.M., Holloway, J.W., Yoshisue, H., Pang, Y.Y., Cakebread, J. and Davies, D.E., The genetics of asthma: ADAM33 as an example of a susceptibility gene. *Proc Am Thorac Soc*, 3:440-443, 2006.
46. Sampsonas, F., Kaparianos, A., Lykouras, D., Karkoulias, K. and Spiropoulos, K., DNA sequence variations of metalloproteinases: their role in asthma and COPD. *Postgrad Med J*, 83:244-250, 2007.
47. Sadeghnejad, A., Ohar, J.A., Zheng, S.L., Sterling, D.A., Hawkins, G.A., Meyers, D.A. and Bleecker, E.R., Adam33 polymorphisms are associated with COPD

and lung function in long-term tobacco smokers. *Respir Res*, 10:21, 2009.
48. Kew, R.R. and Webster, R.O., Gc-globulin (vitamin D-binding protein) enhances the neutrophil chemotactic activity of C5a and C5a des Arg. *J Clin Invest*, 82:364-369, 1988.
49. Ito, I., Nagai, S., Hoshino, Y., Muro, S., Hirai, T., Tsukino, M. and Mishima, M., Risk and severity of COPD is associated with the group-specific component of serum globulin 1F allele. *Chest*, 125:63-70, 2004.
50. Schellenberg, D., Pare, P.D., Weir, T.D., Spinelli, J.J., Walker, B.A. and Sandford, A.J., Vitamin D binding protein variants and the risk of COPD. *Am J Respir Crit Care Med*, 157:957-961, 1998.
51. Sandford, A.J., Joos, L. and Pare, P.D., Genetic risk factors for chronic obstructive pulmonary disease. *Curr Opin Pulm Med*, 8:87-94, 2002.
52. Hersh, C.P., Demeo, D.L., Lange, C., Litonjua, A.A., Reilly, J.J., Kwiatkowski, D., Laird, N., Sylvia, J.S., Sparrow, D., Speizer, F.E., Weiss, S.T. and Silverman, E.K., Attempted replication of reported chronic obstructive pulmonary disease candidate gene associations. *Am J Respir Cell Mol Biol*, 33:71-78, 2005.
53. Klein, W., Rohde, G., Arinir, U., Hagedorn, M., Durig, N., Schultze-Werninghaus, G. and Epplen, J.T., A promotor polymorphism in the Interleukin 11 gene is associated with chronic obstructive pulmonary disease. *Electrophoresis*, 25:804-808, 2004.
54. Dinarello, C.A., IL-1: discoveries, controversies and future directions. *Eur J Immunol*, 40:599-606, 2010.
55. Joos, L., McIntyre, L., Ruan, J., Connett, J.E., Anthonisen, N.R., Weir, T.D., Pare, P.D. and Sandford, A.J., Association of IL-1beta and IL-1 receptor antagonist haplotypes with rate of decline in lung function in smokers. *Thorax*, 56:863-866, 2001.
56. van der Pouw Kraan, T.C., Kucukaycan, M., Bakker, A.M., Baggen, J.M., van der Zee, J.S., Dentener, M.A., Wouters, E.F. and Verweij, C.L., Chronic obstructive pulmonary disease is associated with the -1055 IL-13 promoter polymorphism. *Genes Immun*, 3:436-439, 2002.
57. Hu, R.C., Xu, Y.J. and Zhang, Z.X., [Study on the correlation of interleukin-13 polymorphism and susceptibility to chronic obstructive pulmonary disease in Chinese Han population]. *Zhonghua Liu Xing Bing Xue Za Zhi*, 25:607-611, 2004.
58. Morris, D.G., Huang, X., Kaminski, N., Wang, Y., Shapiro, S.D., Dolganov, G., Glick, A. and Sheppard, D., Loss of integrin alpha(v)beta6-mediated TGF-beta activation causes Mmp12-dependent emphysema. *Nature*, 422:169-173, 2003.
59. Silverman, E.K., Mosley, J.D., Palmer, L.J., Barth, M., Senter, J.M., Brown, A., Drazen, J.M., Kwiatkowski, D.J., Chapman, H.A., Campbell, E.J., Province, M.A., Rao, D.C., Reilly, J.J., Ginns, L.C., Speizer, F.E. and Weiss, S.T., Genome-wide linkage analysis of severe, early-onset chronic obstructive pulmonary disease: airflow obstruction and chronic bronchitis phenotypes. *Hum Mol Genet*, 11:623-632, 2002.
60. Suthanthiran, M., Li, B., Song, J.O., Ding, R., Sharma, V.K., Schwartz, J.E. and 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*, 97:3479-3484, 2000.
61. Celedon, J.C., Lange, C., Raby, B.A., Litonjua, A.A., Palmer, L.J., DeMeo, D.L., Reilly, J.J., Kwiatkowski, D.J., Chapman, H.A., Laird, N., Sylvia, J.S., Hernandez, M., Speizer, F.E., Weiss, S.T. and Silverman, E.K., The transforming growth factor-beta1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD). *Hum Mol Genet*, 13:1649-1656, 2004.
62. Lech-Maranda, E., Baseggio, L., Bienvenu, J., Charlot, C., Berger, F., Rigal, D., Warzocha, K., Coiffier, B. and Salles, G., Interleukin-10 gene promoter polymorphisms influence the clinical outcome of diffuse large B-cell lymphoma. *Blood*, 103:3529-3534, 2004.
63. Ogawa, Y., Duru, E.A. and Ameredes, B.T., Role of IL-10 in the resolution of airway inflammation. *Curr Mol Med*, 8:437-445, 2008.
64. Moore, K.W., de Waal Malefyt, R., Coffman, R.L. and O'Garra, A., Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol*, 19:683-765, 2001.
65. Takanashi, S., Hasegawa, Y., Kanehira, Y., Yamamoto, K., Fujimoto, K., Satoh, K. and Okamura, K., Interleukin-10 level in

sputum is reduced in bronchial asthma, COPD and in smokers. *Eur Respir J*, 14:309-314, 1999.
66. Barnes, P.J. and Stockley, R.A., COPD: current therapeutic interventions and future approaches. *Eur Respir J*, 25:1084-1106, 2005.
67. Kurreeman, F.A., Schonkeren, J.J., Heijmans, B.T., Toes, R.E. and Huizinga, T.W., Transcription of the IL10 gene reveals allele-specific regulation at the mRNA level. *Hum Mol Genet*, 13:1755-1762, 2004.
68. Turner, D.M., Williams, D.M., Sankaran, D., Lazarus, M., Sinnott, P.J. and Hutchinson, I.V., An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet*, 24:1-8, 1997.
69. He, J.Q., Shumansky, K., Zhang, X., Connett, J.E., Anthonisen, N.R. and Sandford, A.J., Polymorphisms of interleukin-10 and its receptor and lung function in COPD. *Eur Respir J*, 29:1120-1126, 2007.
70. Miteva, L. and Stanilova, S., The combined effect of interleukin (IL)-10 and IL-12 polymorphisms on induced cytokine production. *Hum Immunol*, 69:562-566, 2008.
71. Demeo, D.L., Campbell, E.J., Barker, A.F., Brantly, M.L., Eden, E., McElvaney, N.G., Rennard, S.I., Sandhaus, R.A., Stocks, J.M., Stoller, J.K., Strange, C., Turino, G. and Silverman, E.K., IL10 polymorphisms are associated with airflow obstruction in severe alpha1-antitrypsin deficiency. *Am J Respir Cell Mol Biol*, 38:114-120, 2008.
72. Kim, J.M., Brannan, C.I., Copeland, N.G., Jenkins, N.A., Khan, T.A. and Moore, K.W., Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes. *J Immunol*, 148:3618-3623, 1992.
73. Stanilova, S.A., Miteva, L.D., Karakolev, Z.T. and Stefanov, C.S., Interleukin-10-1082 promoter polymorphism in association with cytokine production and sepsis susceptibility. *Intensive Care Med*, 32:260-266, 2006.
74. Lyon, H., Lange, C., Lake, S., Silverman, E.K., Randolph, A.G., Kwiatkowski, D., Raby, B.A., Lazarus, R., Weiland, K.M., Laird, N. and Weiss, S.T., IL10 gene polymorphisms are associated with asthma phenotypes in children. *Genet Epidemiol*, 26:155-165, 2004.
75. Burgess, J.L., Fierro, M.A., Lantz, R.C., Hysong, T.A., Fleming, J.E., Gerkin, R., Hnizdo, E., Conley, S.M. and Klimecki, W., Longitudinal decline in lung function: evaluation of interleukin-10 genetic polymorphisms in firefighters. *J Occup Environ Med*, 46:1013-1022, 2004.
76. Seifart, C., Dempfle, A., Plagens, A., Seifart, U., Clostermann, U., Muller, B., Vogelmeier, C. and von Wichert, P., TNF-alpha-, TNF-beta-, IL-6-, and IL-10-promoter polymorphisms in patients with chronic obstructive pulmonary disease. *Tissue Antigens*, 65:93-100, 2005.
77. Trajkov, D., Mirkovska-Stojkovikj, J., Petlichkovski, A., Strezova, A., Efinska-Mladenovska, O., Sandevska, E., Sibinovska, O., Hristomanova, S., Djulejic, E., Petrov, J., Gogusev, J. and Spiroski, M., Association of cytokine gene polymorphisms with chronic obstructive pulmonary disease in Macedonians. *Iran J Allergy Asthma Immunol*, 8:31-42, 2009.
78. Hu, R.C., Xu, Y.J., Zhang, Z.X. and Xie, J.G., [Polymorphism of interleukin-10 gene promoter and its association with susceptibility to chronic obstructive pulmonary disease in Chinese Han people]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*, 20:504-507, 2003.
79. Dimov, D., Vlaykova, T., Miteva, L., Ilieva, V., Koychev, A., Angelova, M., Prakova, G. and Stanilova, S., [Investigation on the role of -1082A/G IL-10 polymorphism as risk factor for COPD.] [In Bulgarian]. *Torakalna Medicina*, (in press), 2010.