REVIEW

DNA sequence variations of metalloproteinases: their role in asthma and COPD

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Asthma and chronic obstructive pulmonary disease (COPD) are complex genetic diseases that cause considerable morbidity and mortality worldwide. Genetic variability interacting with environmental and ethnic factors is presumed to cause tobacco smoke susceptibility and to influence asthma severity. A disintegrin and metalloproteinase 33 (ADAM33) and matrix metalloproteinase-9 (MMP9) appear to have important roles in asthma and COPD pathogenesis. ADAM33 and MMP9 genetic alterations could possibly contribute to the establishment and progression of these multifactorial diseases, although their association with the clinical phenotypes has not yet been elucidated. However, the occurrence of these alterations does not always result in clear disease, implying that either they are an epiphenomenon or they are in proximity to the true causative alteration. This review summarises the most recent literature dealing with the genetic variations of metalloproteinases and outlines their potential pathogenetic outcome.

Asthma is a rapidly growing public health problem affecting about 300 million people worldwide. It accounts for 1 in every 250 deaths[1] with an approximate economic burden of $12.7 billion in the United States.[2] Moreover, the prevalence of asthma and related mortality has risen over the last 30 years,[3 4] with racial[5] and urban[6] predisposition recognised. Chronic obstructive pulmonary disease (COPD) is also a growing public health problem and has been identified as the fourth leading cause of morbidity and mortality and will rise globally to the third by the year 2020.[7 8] Tobacco smoke is the predominant risk factor for the development of COPD.[8–11] In the USA, COPD is apparent in 12–13% of the general population.[11]

The ''Dutch hypothesis''[12] stated that asthma and COPD are the two extremes of a heterogeneous obstructive phenotype, ''the chronic nonspecific lung disease''. Nevertheless, numerous dissimilarities exist in the clinical presentation and pathogenetic mechanisms of these two diseases. Despite these differences, novel studies involving metalloproteinases (mainly matrix metalloproteinase-9 (MMP9) and a disintegrin and metalloproteinase 33 (ADAM33)) showed that they are undoubtedly involved in the pathogenesis of COPD and asthma, but the precise mechanism has not yet been elucidated.

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ADAM33 AND ASTHMA RELATED PHENOTYPES

ADAM33 is a zinc-dependent endopeptidase, with pro-domain, catalytic, disintegrin-like, cysteine rich and epidermal growth factor-like domains (fig 1). It is abundantly expressed in smooth muscle containing organs, in asthmatic subepithelial fibroblasts (under the respiratory epithelium)[13 14] but not in respiratory epithelium or non-mesenchymal haematopoietic cells.[13] Expression within the structural cells of the airway wall is consistent with the hypothesis that ADAM33 is involved in airway hyper-responsiveness, an asthma linked phenotype.[13] Interestingly, various ADAM33 isoforms exist in human embryonic bronchi and surrounding mesenchyme, indicating its contribution to smooth muscle development and function. Additionally, its presence in mesenchymal tissues is thought to cause ''unusual'' airway formation that leads to the origins of asthma in early life.[15] ADAM33 expression increases during lung development and growth, but simple up- or down-regulation of its expression levels is not likely to account for its involvement in asthma.[15] Thus, contrary to the general belief that remodelling in asthma is secondary to long-standing inflammation, this process starts early in life, with pathological features present before onset of disease. Therefore, a major consideration arises: does asthma lead to non-reversible airway obstruction and COPD related phenotypes in childhood, with clinical outcome becoming apparent during adulthood?

The structure of the protease/cleavage domain of ADAM33 is dominated by amino acids with uncharged side chains, such as valine/serine or leucine/alanine, and is unfavourable to amino acid substitutions, exhibiting decreased catalytic activity.[16] Thus, all single nucleotide polymorphisms (SNPs) potentially modulating the catalytic domain of ADAM33 can contribute to asthma pathogenesis (fig 1).

Association of genetic variations of ADAM33 and asthma related phenotypes

Genome-wide scans and analysis of 135 SNPs in 23 genes among individuals from the UK and USA, revealed that the ADAM33 region was significantly

Abbreviations: ADAM33, a disintegrin and metalloproteinase 33; BHR, bronchial hyperresponsiveness; COPD, chronic obstructive pulmonary disease; ECM, extracellular matrix; LD, linkage disequilibrium; MMP, matrix metalloproteinase; SNP, single nucleotide polymorphism

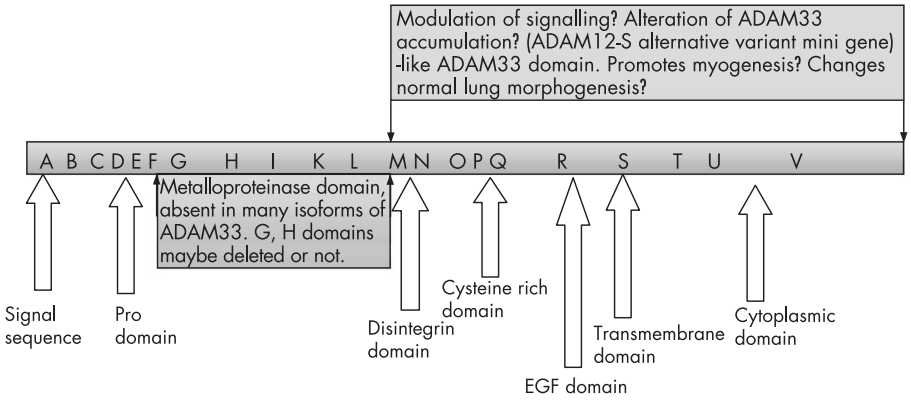

Figure 1 Simplified structure of ADAM33 and its exons.

related to asthma, which was more apparent when the asthma plus bronchial hyper-responsiveness (BHR) phenotype was examined. Significant SNPs in case control studies were: L_1 (Ala359Val), V4 (exonic G/C), V1 (exonic A/T), V-1 (-32 C/A), T1 (12433 T/C, Met764Thr), S1 (G/A exon 19, Val to Iso), S2 (10918 G/C, Gly717Gly) and T2 (12462 C/T, Pro774Ser). Numerous haplotypic analyses of these SNPs confirmed and strengthened the correlation between asthma and ADAM33.[17]

This original description of ADAM33 as an ''asthma'' gene was followed by many studies that examined different ethnic groups, revealing controversial results. In family and case control based studies among Latin populations, the Genetics of Asthma in Latino Americans (G.A.L.A.) study (Puerto Rican and Mexican groups) failed to reveal any association between asthma and related phenotypes (bronchodilator responsiveness, asthma severity, IgE levels) and V4, V1, V-1 , T1 , S1 and S2 SNPs.[18] Intriguing data from other researches demonstrated that, among Hispanic individuals, S2 and T2 SNPs were related to asthma, whereas IgE levels were associated with T1 and T2 SNPs.[19]

The Childhood Asthma Management Program (CAMP), a multi-centre clinical trial, disclosed that in a Hispanic ethnic group T1 and T+1 (12540 C/T, intron 20) SNPs were marginally linked to blood eosinophil levels and serum IgE.[20]

Case control and family based studies followed the original research that involved Caucasian subjects[17] in an effort to estimate specific genetic changes and their outcome. We can infer that asthma and asthma related phenotypes (asthma plus BHR and total IgE, skin test responsiveness) are linked to ST+4 (11434 C/A, intron 19),[21] ST+5 (intronic C/T),[21] ST+7 (intronic A/G),[19 21 22] V4,[19] T1, T2[19] and F+1 (7575 G/A intron 6)[21 22] SNPs.

African-American cohorts of asthmatic families were also evaluated for ADAM33 variations and haplotypes.[19 20] There was a higher frequency of the S2 SNP and numerous haplotypes among those with asthma.[19] A Korean cohort of patients exhibited a link between BHR, as revealed by a PC_{20} (provocative concentration dose of metacholine producing a 20% fall in FEV_1) of <10 mg/ml of metacholine, and T1 SNP or haplotype 4 (GCGG)[23] (table 1).

Simpson *et al* recently reported that 3-year-old carriers of the rare ADAM33 allele F+1 (intronic G/A) had reduced lung function. In addition, other SNPs were also associated with lower FEV_1: M+1 (intronic A/C), T1 and T2. He proposed that ADAM33 is a ''lung function gene'' which is able to modulate the architecture of the lung leading to remodelled airways and an obstructive pattern in early life that persists into adulthood (COPD-like phenotype).[24] Among patients with asthma, a recent genetic study disclosed an association between the S_2 SNP and longitudinal FEV_1 decline. According to the authors, this SNP may modify the outcome of asthma leading to irreversible airway obstruction (COPD-like phenotype) associated with increased symptomatology, including exacerbations and loss of quality of life.[25] Correlation of lung function decline and ADAM33 polymorphisms in the general, non-asthmatic population showed that individuals homozygous for S_2 and Q-1 (intronic C/T) and heterozygous for S_1 SNPs had accelerated decline in FEV_1. Individuals with COPD had a higher prevalence of the SNPs F+1, S_1, S_2 and T_2. Haplotypes that

Table 1 Genetic variation of ADAM33 in different ethnic groups

Caucasian	Latin	African-American	Korean
L_1 (Ala359Val)[17]	S2	S2	T1
V4 (exonic G/C)[17]	T2		Haplotype 4 (GSGG)[23]
V1 (exonic A/T)[17]	T1		
V-1 (-32 C/A)[17]	T2		
T1 (12433 T/C, Met764Thr)[17]	T+1 (12540 C/T)		
S1 (G/A exon 19, Val to Iso)[17]			
S2 (10918 G/C, Gly717Gly)[17]			
T2 (12462 C/T, Pro774Ser)[17]			
ST+4 (11434 C/A, intron 19)[21]			
ST+5 (intronic C/T)[21]			
ST+7 (intronic A/G)[19 21 22]			
V4,[19] T1, T2[19]			
F+1 (7575 G/A intron 6)[21 22] SNPs			

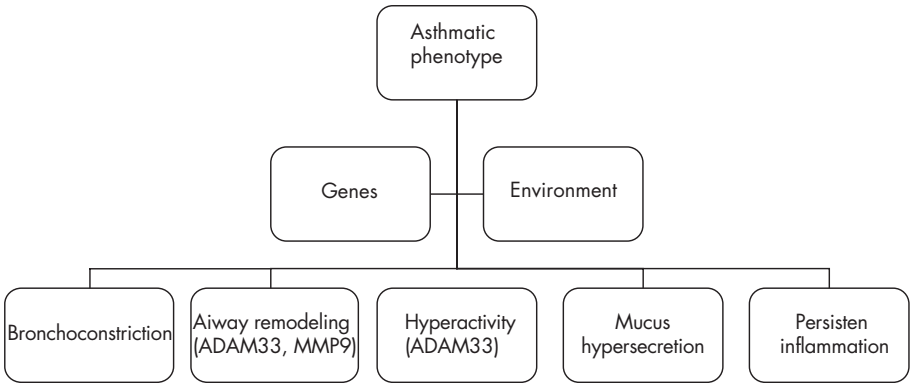

Figure 2 Involvement of ADAM33 and MMP9 in the asthmatic phenotype.

include at least one minor allele for F+1, Q-1, S_1, S_2 and V_4 were associated with COPD.[26] Noguchi *et al* demonstrated that minor alleles of S1, ST14 and T2 SNPs were over-transmitted to asthma-affected offspring. All cases were of childhood or child-onset asthma, supporting the hypothesis that ADAM33 is responsible for narrower airways.[27]

Whole gene scanning of ADAM33 revealed numerous new and previously recognised SNPs located in critical regions, such as the promoter and catalytic domains.[28] The -2154G/A SNP in the promoter region may alter the gene's transcriptional rate.[28] Nevertheless, the F1 SNP is close to Cys179 (cysteine switch), potentially modulating proteolytic activity[16] and the L_1 is located in the catalytic section of ADAM33,[16] whereas the T2 and S2 SNPs may also affect signalling, being located within the putative SH3-binding domain.[16] The Q-1 SNP is located near the EGF domain and can modulate lung morphogenesis[28] (fig 1). Nevertheless, all the above polymorphisms of ADAM33 potentially influence its function, since no evidence exists for their involvement in the pathogenesis of the disease.

It is well documented that ADAM33 is mainly retained in fibroblast nuclei. Thus SNPs modulating the localisation of the molecule, and not just up- or down-regulation of its expression levels, may influence its involvement in asthma or COPD pathogenesis.[14 15] Additionally, splice variants of the ADAM33 gene, lacking the PRO/metalloproteinase domains (fig 1) that retain the downstream domains, are of similar composition to the ADAM12-S alternative variant mini gene that was shown to induce in vivo myogenesis, a characteristic pathological finding of asthmatic bronchi.[14] Asthmatic myofibroblasts have the unique ability to proliferate in the absence of exogenous growth factors,[29] thus ADAM33, or its splicing variants, could serve as an endogenous growth factor in individuals with asthma.

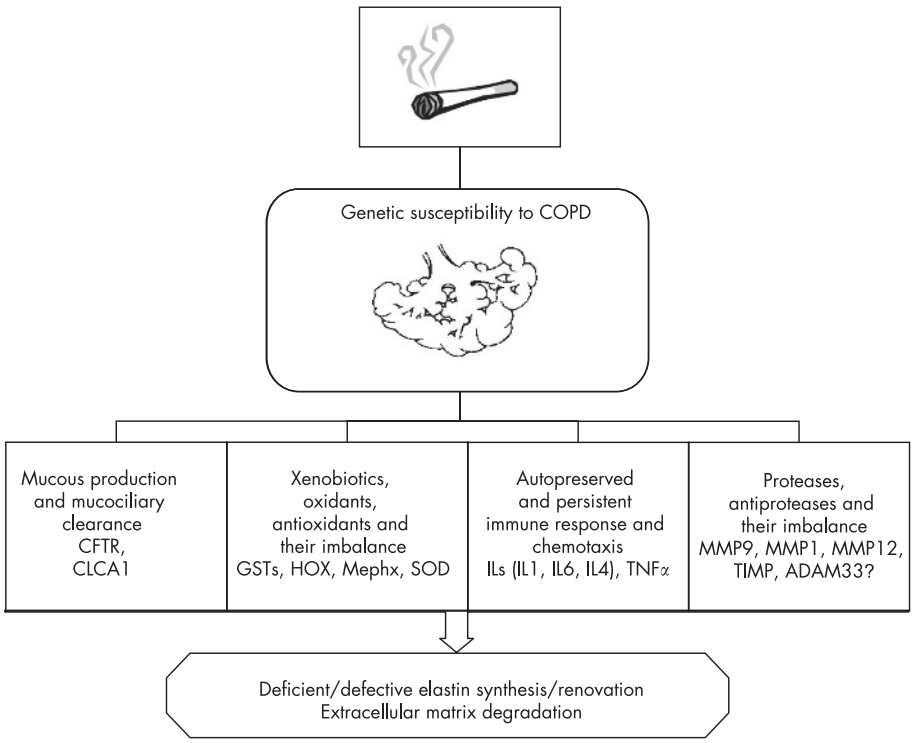

Figure 3 Components of genetic pathogenesis of COPD. ADAM33, a disintegrin and metalloproteinase 33; CFTR, cystic fibrosis transmembrane regulator; CLCA1, human calcium-activated chloride channel 1; GST, glutathione S-transferase; HOX, haeme oxygenase; IL, interleukin; mEPHX, human microsomal epoxide hydrolase; SOD, superoxide dismutase; TIMP, tissue inhibitor of metalloprotease.

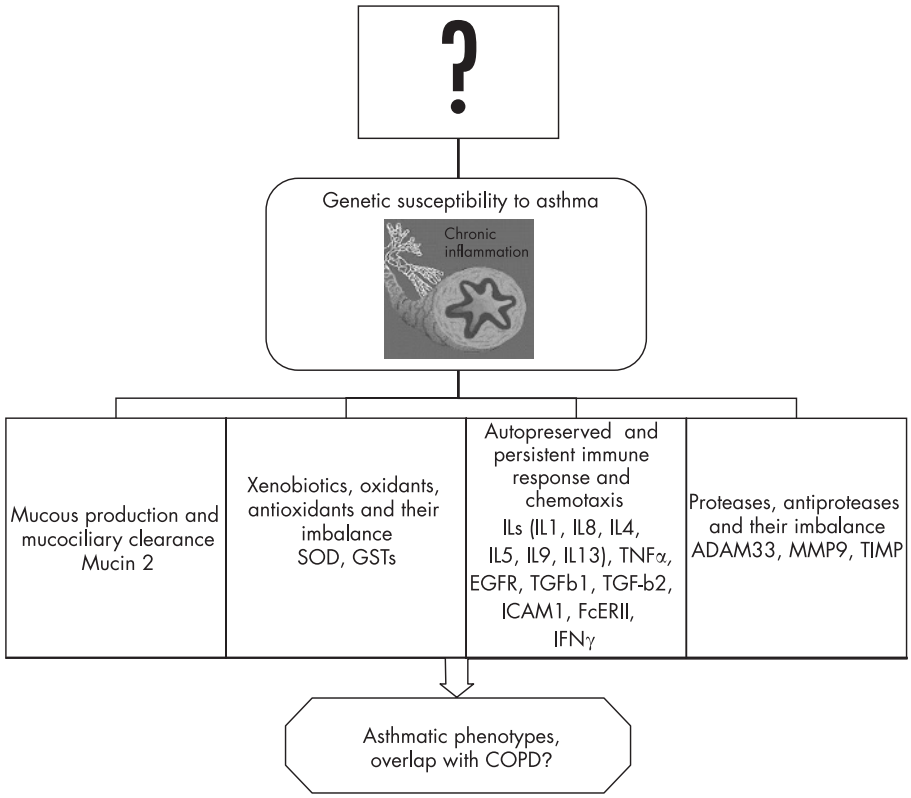

Figure 4 Components of genetic pathogenesis of asthma. GST, glutathione S-transferase; IL, interleukin; SOD, superoxide dismutase; TIMP, tissue inhibitor of metalloprotease.

THE ROLE OF MATRIX METALLOPROTEINASES IN PULMONARY DISEASES

Matrix metalloproteinases (MMPs) are structurally related endopeptidases which have enzymatic activity to digest a range of proteins involved in pulmonary morphology, cell movement and recruitment of inflammatory cells in the lung. In addition to COPD and asthma, MMPs are involved in numerous pulmonary pathologies, such as lung cancer, acute respiratory distress syndrome, sarcoidosis, tuberculosis, silicosis, cystic fibrosis, pneumonia and bronchiolitis obliterans.[30 31] Their elastolytic activity is also curtailed in lung fibrosis, leading to a microenvironment unfavourable to collagenolytic activity.[31]

MMP9 and asthma

MMP9 is up-regulated in the lung during inflammation, especially in leukocytes. The major function of lung gelatinases during allergic inflammation is facilitating egression of recruited inflammatory cells through the airway. MMP9 is the dominant airway MMP, controlling trafficking of inflammatory cells.[32] Increased amounts of MMP9 in bronchoalveolar lavage fluid from patients with asthma[33] and its ability to control T cell responses to allergens[34] highlight its possible significance in the pathogenesis of asthma. Many MMP9 SNPs were therefore evaluated for their contribution to the asthma phenotype, for example -1562 C/T[35 36] +6C/T,[36] Arg279Gly (+836G/A), -1702 T/A,[36 37] -861 C/T[37] SNPs, and the subsequent haplotypes, but no association was demonstrated.[36]

Intriguingly, the -1562C/T SNP is a functional polymorphism that modulates the binding DNA-site of a repressor protein, decreasing the protein–DNA interaction, and leads to higher promoter activity of the T allele and consequently to higher protease activity.[38 39] Moreover, the abovementioned polymorphisms Arg279Gly (+836G/A) and -1702 T/A are located in the catalytic domain of the MMP9 domain (within one of the fibronectin type-II-like repeats required for binding to elastin) and next to polyomavirus enhancer A binding protein 3, respectively.[39] Stressing the importance of the -1562C/T SNP, designated as -1590C/T, luciferase reporter activity, after

Table 2 Major clinical differences between asthma and COPD

	Asthma	COPD
Airflow limitation	Reversible unless remodelling present	Mainly irreversible
Bronchial hyper-responsiveness	Increased	Variable
Lung tissue integrity	Not affected	Major tissue degradation
Treatment response	Significant to corticosteroids	Minor
Clinical consequences of exacerbations	Usually minor	Mostly decreased Lung function

Table 3 Major immunological and inflammatory differences between asthma and COPD

Asthma	COPD
Eosinophils	Neutrophils
Mast cells	Macrophages
CD4+	CD8+
Increased CD4+/CD8+	Decreased CD4+/CD8+
RANTES (↑ in mild asthma)	RANTES (↑ ↑ ↑ in exacerbation)
Effective apoptotic cell removal (?)	Defective apoptotic cell removal

RANTES, Regulated on Activation, Normal T cells Expressed and Secreted.

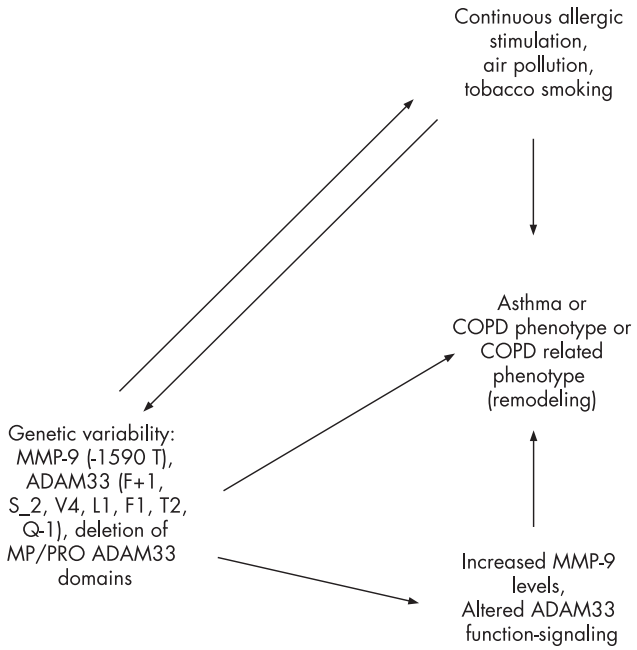

Figure 5 MMP9 and ADAM33 in COPD and asthma.

degradation by MMP9, was found to be 3.48-fold higher in subjects with asthma carrying the T allele. This SNP was in complete linkage disequilibrium (LD) with 2127G/T SNP, which was associated with childhood atopic asthma.[40] The susceptible allele possibly contributes to enhanced MMP9 production during exacerbations, leading to the already recognised increased activity and levels of MMP9 during exacerbations of asthma.[41]

Genetic variations of MMPs and COPD

MMP1, MMP2, MMP8, MMP9 and MMP12[30 42–46] were involved in COPD pathogenesis[47] and extracellular matrix (ECM) remodelling.[42] Neutrophils, macrophages, mononuclear inflammatory cells,[46] dendritic cells[48] and type II pneumocytes[43] secrete vast amounts of extremely active MMPs.[43 44 49]

MMP9 -1562C/T SNP, in the promoter region of the gene, was a significant risk factor for the development of emphysema apparent on conventional chest CT scans,[50] by spirometric values[50 51] or high resolution CT,[52] while lung function tests did not show any difference between patients with the C allele and those with the T allele.[52] Considering that the particular SNP augments MMP9 transcription, we can speculate that it is a COPD causing SNP.

Various others SNPs of different MMPs genes were examined for possible contribution to the COPD phenotype: MMP1 G-1607 G/GG (additional guanine-G), MMP9 (CA-repeat), MMP12 (-82A/G) and MMP12 (Asn357Ser),[53] since they probably increase the transcriptional rate of the respective genes or modulate their catalytic traits. The activity of the promoter of MMP9 depends on the length of d(CA) repeats. Twenty one d(CA) repeats are required for basal activity of the MMP9 promoter.[54] The frequency of the MMP1-1607G allele was increased in a group of patients with fast lung function decline, despite the fact that it is related to normal or even decreased MMP1 expression,[53] probably due to another allele in LD with it. The additional G creates an Ets binding site, displaying higher transcriptional rates in normal fibroblasts, leading to aggressive matrix degradation.[55] Nevertheless, this study[53] did not reveal any associations between the other SNPs and COPD, albeit they were presumed to cause higher transcription rates. Interestingly, the MMP1 1607 G in association with the MMP12 Ser357 (Asn357Ser substitution) was increased in the group with fast lung function decline.[53] The Asn357Ser substitution potentially alters the catalytic activity of the protein.[53] All the aforementioned SNP-based association studies reveal the significance of this approach but also highlight the difficulty of appropriate SNP selection. This can be partially overcome by haplotypic analysis of candidate SNPs, identifying more sequence variants at biologically functional loci.

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IS THERE A GENETIC AND CLINICAL OVERLAP BETWEEN ASTHMA AND COPD AND WHAT IS THE ROLE OF MMP9 AND ADAM33?

The perception that asthma and COPD are the two extremes of a common obstructive phenotype was initially granted as the Dutch hypothesis. COPD and asthma phenotypes have clinical and genetic peculiarities which share differences and similarities both supporting and opposing the aforementioned hypothesis (tables 2 and 3; figs 2–4).

MMP9 and ADAM33 confer a pathogenetic route where asthma can lead to a COPD-like phenotype. MMP9 was thought to cause airway remodelling and collagen deposition, consequent to incessant allergen exposure, since its production was locally up-regulated in a mouse model of allergic asthma.[56] Lung tissue can be injured by MMP9 and later remodelled, even in the early phases of allergen exposure.[57] Moreover, COPD was associated with increased MMP9 levels and functional SNP of the MMP9 promoter leading to increased transcriptional rates that may stimulate smooth muscle cell migration.[38]

Additionally, many studies suggested ADAM33 and its genetic alterations cause early airway remodelling and loss of quality of life.[24–27] The consistent genetic association of these SNPs, mainly F+1 and S_2, with rapid FEV_1 decline, narrower airways, irreversible airway obstruction and remodelling across these studies supports the possibility of their involvement in the development of a COPD-like phenotype, considering that 7% of non-smokers develop the disease.[11]

Irreversible airway obstruction is a COPD-like phenotype frequently seen in patients with asthma.[58] Intriguingly, not all of those with asthma develop airway remodelling, similar to the fact that not all smokers develop COPD (less than 20%).

Moreover, some with well treated asthma develop increased lung function decline and irreversible airway obstruction for no apparent reason.[58] Therefore, the functional SNPs of MMP9 and ADAM33 may permanently affect the anatomy of the lung, reflecting the hostile gene–environment interaction (fig 5).

PERSPECTIVES

ADAM33 and MMPs are clearly important enzymes in normal and abnormal processes involved in ECM remodelling and degradation. Increased MMP9 activity appears to be a necessary part of COPD development, in association with other components of COPD pathogenesis. ADAM33 is probably responsible for airway remodelling and BHR even in the earliest years of life. Genetic heterogeneity found throughout these studies implies that asthma is caused by various mutations in the same gene. On the other hand, different LD patterns among different populations reveal that untyped causative factors are probably the same, but the assortment of SNPs in strong LD with the variant differs among ethnic groups.

Future research dealing the genetics of asthma and COPD should evaluate the genetic background of patients with a history of asthma that develops into irreversible airway obstruction, a COPD-like phenotype.

However, all these basic science studies that elucidate genetic alterations would have little meaning for physicians if we could not take advantage of this knowledge in a clinical setting. The genetic profile of each individual and the consequent pattern of enzymatic expression can lead us to a prompt diagnosis of potential asthma severity, airway remodelling, and gradually and rapidly escalating COPD development, in smokers and non-smokers. It is already well established that subjects with increased decline in lung function, even with normal baseline function tests, exhibit greater risk of death and need for hospital care.[59] This will benefit all candidate patients and health care providers.

MULTIPLE CHOICE QUESTIONS (TRUE (T)/FALSE (F) ANSWERS AFTER THE REFERENCES)

1. What proportion of smokers develop COPD?

   A. Less than 10%
   B. 10–20%
   C. 20–30%
   D. 30–40%

2. Childhood asthma may lead to irreversible airway obstruction and COPD related phenotypes in what proportion of those with asthma?

   A. Less than 20%
   B. 20–40%
   C. 40–60%
   D. 60–80%

3. When does MMP-9 induce lung injury and remodelling?

   A. In the early stages of allergen exposure
   B. In the late stages of allergen exposure

4. Is a long-standing inflammation is necessary for lung remodelling?

   A. Yes
   B. No

5. Does functional genetic alteration always lead to altered enzymatic activity and disease development?

   A. Yes
   B. No

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ANSWERS

1. (A) F, (B) T, (C) F, (D) F.
2. (A) T, (B) F, (C) F, (D) F.
3. (A) T, (B) F.
4. (A) F, (B) T.
5. (A) F, (B) T.