Genetic susceptibility

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Synopsis

Why only 20% of smokers develop clinically relevant COPD was a puzzle for many years. Now, epidemiological studies point clearly towards a large heritable component. The combination of genome wide association studies and candidate gene analysis is helping to identify those genetic variants responsible for an individual's susceptibility to developing COPD. In this review, we will examine the current data implicating specific loci and genes in the pathogenesis of COPD.

Introduction

Although most smokers will die of a smoking-related disorder, only 20% suffer from significant COPD. Familial clustering suggests that heritable factors play an important role in the development of this disease [1,2]. In one large series, 18.6% of population-attributable risk for COPD could be accounted for by family history; patients with an affected parent having more severe disease, more frequent exacerbations and a worse quality of life [3]. Similarly, in twin pairs the risk of developing COPD is higher for monozygotic than for dizygotic twins, with 60% of the individual susceptibility explained by genetic factors [4]. Both the airway and emphysema components cluster independently suggesting that different genetic factors play a role in the development of these two components of the disease [5]. Unsurprisingly, for a disorder with a substantial heritable component, race and ethnicity appear to impact upon the development of COPD. For example, in the COPDGene Study, 42% of affected African Americans were found to suffer severe early onset COPD (age < 55 years, FEV1 < 50% predicted) compared with 14% of non-Hispanic whites [6]. In contrast, self-reported Hispanic ethnicity or Native American genetic ancestry have both been reported to be associated with significantly lower risks of developing COPD [7].

Efforts to identify the genetic determinants of COPD have evolved as the available technologies have changed. The analysis of candidate genes yielded some successes that will be discussed later, but that approach also led to numerous blind alleys, with initial excitement followed by disappointment as associations proved impossible to reproduce. The analysis of large cohorts of patients in genome-wide association studies (GWAS) using microarray technology to assay up

to a million single nucleotide polymorphisms (SNPs) in each case led to the unbiased identification of novel disease associated loci. This approach is hypothesis-free and so has the potential to open novel avenues of research.

Unbiased approaches

Until recently, only mutations of the *SERPINA1* gene that are responsible for α_1-antitrypsin deficiency were unambiguously linked with the development of COPD. However, this disorder accounts for only 1-2% of cases of COPD and so other disease-associated alleles must exist. Recent large multinational GWAS have shed much light on this. In addition to validating the involvement of some candidate genes previously suspected of playing a role in the pathogenesis of COPD, these landmark studies have identified novel pathways that might plausibly lead to novel therapies for COPD.

SNPs in chromosome 15 at the α-nicotinic acetylcholine receptor *CHRNA3/5* locus (15q25.1; rs8034191 and rs1051730) were found to reach genome-wide significance and have subsequently been replicated in several independent studies [8-13]. This locus is significantly associated with pack-years of smoking, emphysema (by CT), and airflow obstruction [9,14]. Notably, the C allele of the rs8034191 SNP was estimated to have a population attributable risk for COPD of 12.2% and has previously been identified in genome–wide association studies of lung cancer, being thought to be important in nicotine addiction [15]. Individuals who carry this SNP may require more cigarettes to satisfy nicotine addiction, may inhale more deeply and may find it more difficult to withdraw from cigarette smoking. Indeed, it has been reported that the association of the *CHRNA3/5* locus is substantially mediated by

smoking phenotype [12], although this finding has been disputed [14]. However, *IREB2* is a gene in tight linkage disequilibrium with *CHRNA3/5* and has also been identified as a potential determinant of COPD [9,16,17]. This gene encodes an iron regulatory protein localised in epithelia that may plausibly affect oxidative stress responses in smoked-exposed lungs. These candidate genes are not mutually exclusive and it remains possible that both genes within the haplotype contribute to the disease phenotype [12].

GWAS have also identified a locus at 4q22.1 containing the gene *FAM13A* to be significantly associated with COPD and lung function in multiple cohorts [9,18]. While the function of FAM13A is unclear, another gene at 4q31 newly identified and replicated by GWAS as being associated with both COPD and lung function, encodes hedgehog interacting protein (HHIP), which appears to play a role in signalling that modulates lung development or remodelling [15,19-21]. HHIP is expressed in pulmonary tissues but at lower levels in COPD-affected lungs, and disease-associated SNPs have been identified within the gene's promoter (rs6537296A and rs1542725C) that appear to reduce its transcription [22]. Other loci that have been identified using similar techniques include 2q35, 4q24, 5q33, 6p21, 15q23 and 19q13, although these require validation [20,23]. Other COPD-related phenotypes have also been linked with specific loci, for example low body mass index in COPD is significantly association with SNP rs8050136 within the first intron of the fat mass and obesity-associated (*FTO*) gene [24], while a SNP in *BICD1* (rs10844154 in 12p11.2) is associated with the presence and severity of emphysema on CT scan [25].

Candidate gene approaches

The extra-cellular matrix

Alveolar tissue consists of epithelial cells, capillaries and extra-cellular matrix (ECM), the latter comprising a complex network of scaffolding proteins, principally elastin and collagen (Figure 1). The elastin filaments form from tropoelastin monomers that self assemble into aggregates and then fuse with microfilaments. Multiple covalent cross-links between the lysines in neighbouring filaments provide stability. Cutis laxa is a family of autosomal dominant (OMIM #123700), X-linked (OMIM #304150) and recessive (OMIM #219100, 219200) human diseases characterized by excessively slack connective tissues. Several families with the milder autosomal dominant form show early onset pulmonary pathology including emphysema [26], particularly if inherited with the Z allele of α_1-antitrypsin [27]. Two groups independently identified separate mutations within the ELN (elastin) gene that cause mild cutis laxa and early onset COPD [28,29]. The ELN gene maps to 7q11.23 in man, but as chromosome 7 has not been identified in linkage analysis as a site associated with COPD, it is likely that ELN mutations are a rare cause of this disease.

Elastin fibres bind other proteins including fibulins, which in turn bind multiple ECM components and the basement membrane. The fibulins are a family of 6 proteins, at least 2 of which are mutated in severe autosomal recessive forms of cutis laxa and whose phenotype often includes early onset emphysema [30,31]. A novel mutation in the fibulin-4 gene (FBLN4; 11q13) was recently identified in autosomal recessive cutis laxa with developmental emphysema [30]. The mutation

caused an amino acid substitution in an epidermal growth factor (EGF) like domain of fibulin-4, leading to very low levels of extra-cellular protein. In a consanguineous Turkish family, a homozygous mutation in the related fibulin-5 gene (FBLN5; 14q32.1) was also found to cause cutis laxa and emphysema complicated by recurrent pulmonary infections [31]. Once again, the mutation was located within an EGF-like domain, suggesting these are critical for fibulins to maintain the integrity of the ECM within the lung. Interestingly, analogous mutations in fibrillin, which bares homology to the fibulins, cause Marfan's syndrome. Moreover, mutations of fibrillin (FBN1; 15q21.1) have been described in neonatal Marfan's with very early onset emphysema [32-34].

Menkes disease (OMIM #309400) characterized by abnormal hair and dysmorphic features, is caused by mutations in an intracellular copper transporter (ATP7A; Xq13.3). The clinical features are due to defective connective tissue synthesis believed to be the result of dysfunction of lysyl oxidase. This copper-dependent enzyme is required for proper cross-linking of both collagen and elastin fibres. A recent case report described a child with Menkes disease and severe bilateral pan-lobular emphysema who died aged only 14 months [35]. Gene sequencing revealed a splice-site mutation in ATP7A, suggesting that proper ECM cross-linking is vital for stability of the lung parenchyma.

In contrast to animal models of COPD, mutations in collagen have not been identified in humans. This does not appear to be due to an incompatibility of mutated collagen with survival, as numerous collagen mutations have been described that cause other human diseases. Instead, it may reflect a more important role for elastin integrity in emphysema in humans than in mice. However, aberrant

collagen synthesis has been implicated in COPD. The signaling molecule TGFβ1 enhances collagen synthesis in vivo, and polymorphisms in its gene (TGFB1; 19q13.1) have been associated with COPD [36-40], although a recent, large study found no association between TGFB1 polymorphisms and the rate of lung function decline in smokers [41]. Intriguingly, the TGFβ1 gene maps to a locus on chromosome 19, which has high linkage (LOD 3.3) with FEV_1 in smokers [37,42]. However, as is frequently the case with polymorphism studies, the literature is unclear. For example, two TGFB1 single nucleotide polymorphisms (SNPs), rs1800469 and rs1982073, were found to be independently associated with COPD in two studies [36,39], but in another, they were only significant when analysed as part of a haplotype (combination of alleles), while yet another SNP, rs6957, was significant in its own right [40]. Detailed analysis of the Boston Early-Onset COPD Study data revealed further complexity [37]. While some alleles of TGFB1 were associated with FEV_1 (rs2241712, rs2241718, rs6957), there was a separate but partially overlapping set of alleles associated with airflow obstruction (rs2241712, rs1800469, rs1982073). TGFβ1 protein is inactive when first secreted owing to the presence of an inhibitory N-terminal pro-peptide. It is secreted associated with latent TGFβ1 binding proteins (LTBP), which share structural features with fibrillins, and are assembled into the ECM. Mice with mutations in LTBP4 develop severe emphysema [43]. Intriguingly, the sole study that has addressed LTBP4 (19q13.1-q13.2) polymorphisms found an association with COPD in man [39]. More recently, genome-wide linkage analysis of pedigrees stratified by emphysema status (on CT scan) identified a region on chromosome 1p (LOD score = 2.99) [44]. An intronic SNP in TGFB-recepor-3 at this locus was found to be associated with COPD status, FEV_1 and CT emphysema.

Taken together, these studies provide strong evidence in support of a crucial role for the loss of ECM integrity, in particular the elastic components, in the development of COPD. It is therefore important to consider the enzymes implicated in degradation of the ECM.

Protease-antiprotease balance

The protease anti-protease theory has its roots in the observation that individuals with α_1-antitrypsin are particularly susceptible to COPD and in experimental models of emphysema from the 1960s. This theory suggests that the pathogenesis of COPD and emphysema is the result of an imbalance between enzymes that degrade the ECM within the lung and proteins that oppose this proteolytic activity. Many proteases play important roles in remodeling or inflammation within the lung. It is essential that they be controlled by antiproteases to protect against uncontrolled degradation of the ECM (Figure 2).

The best-understood example of genetically induced emphysema results from mutations in the α_1-antitrypsin gene (*SERPINA1*; 14q32.1). These increase the protein's propensity to form ordered polymers, which are incapable of inhibiting its target enzyme, neutrophil elastase. This abnormal behaviour leads to retention of the protein within hepatocytes as Periodic Acid Schiff positive inclusions and results in plasma deficiency of an important protease inhibitor (OMIM #107400). It is now increasingly recognised that mutant α_1-antitrypsin can also form polymers within the interstitium and alveolar spaces of the lung. These polymers are chemotactic for neutrophils and so combine with the deficiency of α_1-antitrypsin to focus and amplify the inflammatory response within the lung [45]. In most Northern European

populations the frequency of the most severe Z allele is about 1/2000. Classically, Z α_1-antitrypsin homozygotes carry the Glu342Lys mutation and suffer from early onset emphysema when compared to normal MM α_1-antitrypsin individuals. The onset and progression of emphysema is markedly accelerated by cigarette smoking. Moreover, it appears that even a single allele of Z α_1-antitrypsin may increase the risk of COPD. In the longitudinal Copenhagen City Heart Study, the MZ α_1-antitrypsin genotype increased the rate of decline of FEV_1 by 19% compared with those who were MM homozygotes, causing a 30% increased risk of obstructive lung function and a 50% increased risk of physician diagnosed COPD [46]. The authors found that the frequency of the MZ genotype in their Danish population was as high as 5% and so calculated that it would account for 2.4% of cases of COPD. This is in contrast to the ZZ genotype, which was causal in only 0.8% of cases. In meta-analysis, heterozygosity for the Z allele carried an odds ratio for COPD of 2.31 [47]. In one study, the MZ (but not MS; the S allele has the Glu264Val mutation) α_1-antitrypsin genotype was associated with a rapid decline in FEV_1, which was even more marked if there was also a family history of COPD, suggesting an interaction with additional genetic factors [48]. A further meta-analysis combining 17 studies found a 3-fold increase in COPD in SZ α_1-antitrypsin heterozygotes and a small increase in MS α_1-antitrypsin heterozygotes. Other polymorphisms of the SERPINA1 gene do not appear to be associated with increased risk of developing COPD [49].

Other pulmonary serine protease inhibitors may also be involved in the pathogenesis of COPD. Following earlier linkage studies demonstrating an association between chromosome 2q and COPD, expression profiling of genes

within that locus identified *SERPINE2* (2q33-q35) as being up regulated during murine lung development and in the lungs of individuals with COPD [50]. The authors went on to demonstrate an association between SNPs in *SERPINE2* and COPD. *SERPINE2* SNPs were found to segregate with COPD in a large multi-centre family-based study and to be associated with COPD in a case-control analysis [51]. However another large study failed to replicate the association with COPD despite having adequate power [52]. The latter study included individuals with COPD with and without emphysema, while the studies by DeMeo and colleagues [50] included a preponderance of patients with emphysema assessed for lung volume reduction surgery. Nevertheless, while these differences may reflect different COPD phenotypes, they illustrate the need to replicate the findings of genetic association studies in multiple populations before drawing firm conclusions. A recent study of Finish construction workers found that three SNPs within SERPINE2 (rs729631, rs975278, and rs6748795) were in tight linkage disequilibrium and so focused solely on one (rs729631) [53]. This showed a significant association with panlobular emphysema, as seen with mutants of *SERPINA1*.

Since mutations of α_1-antitrypsin so clearly lead to emphysema, one might infer that its target, neutrophil elastase, is central to the pathogenesis of disease. However, mutations in this protease have not been shown to be important, despite being studied extensively in other conditions. Instead, most evidence implicates matrix metalloptroteases (MMPs) in the pathogenesis of COPD. These are zinc-dependent endopeptidases involved in the degradation of many ECM components. A SNP of MMP9 (20q11) was associated with COPD in Japanese [54] and Chinese [55] populations, however a further Japanese study found an association with

emphysema distribution rather than COPD *per se* [56]. Another large study failed to show MMP9 association with COPD, but instead MMP1 (11q22) and MMP12 (11q22) polymorphisms were identified [57]. Further support for a role for MMP9, but not MMP1 polymorphisms, has also been published [58]. Tissue Inhibitors of Metaloproteinases (TIMPs) inhibit the MMPs, but thus far, only one polymorpism in TIMP2 (17q25) has been associated with COPD [59]. When more than 8000 individuals were analysed, the minor allele of the promoter of MMP12 (rs2276109 [-82A-->G]) showed clear association with FEV_1 in a combined analysis of adult ever-smokers and children with asthma and with a reduced risk of the COPD [60]. In a separate study, a haplotype containing this SNP in MMP-12 (rs652438 and rs2276109) was found to be associated with severe COPD (GOLD Stages III and IV) (20078883). When expressed in cells *in vitro*, the COPD-associated A allele of rs652438 was 3-fold more proteolytically active than the G allele suggesting that it might mediate enhanced ECM degradation [61].

Reactive oxygen species

Cigarette smoke contains vast numbers of free radicals that impose an oxidative stress on the lung. Such stress is believed to induce damage through multiple mechanisms, including direct oxidation of cellular lipids and DNA, and through inactivation of key proteins such as α_1-antitrypsin. For this reason, much work has gone in to assessing the role of endogenous antioxidant enzymes in protecting against smoke-induced lung damage.

Many toxins in cigarette smoke are subject to first pass metabolism in the liver. Amongst the many enzymes involved, microsomal epoxide hydrolase (EPHX1;

1q42.1) has been intensely studied in the context of COPD. Several EPHX1 SNPs have been described that affect its activity. One of these leads to a 40% loss of *in vitro* activity (rs1051740 Tyr113His, the "slow" allele), while another increases activity by 25% (rs2234922 His139Arg, the "fast" allele). In 1997, the "slow" variant of EPHX1 was found to increase the risk of emphysema by a staggering odds ratio of 5.0 and of COPD by an odds ratio of 4.1 [62]. Since then, numerous studies have attempted to reproduce this effect with varying success [39,63-74]. Recently, analysis of randomly selected white Danish individuals participating in the Copenhagen City Heart Study (n = 10,038) and the Copenhagen General Population Study (n = 37,022) for the rs1051740 and rs2234922 variants in the *EPHX1* gene combined with a meta-analysis of 19 previous studies indicate that genetically reduced EPHX1 activity is not a major risk factor for COPD or asthma in the Danish population. [75]

Glutathione S-transferase (GST) comprises a large family of enzymes capable of catalyzing the conjugation of reduced glutathione to endogenous and xenobiotic electrophilic compounds. The GSTs are important in the detoxification of many compounds and are highly polymorphic. These polymorphisms have been linked to susceptibility to toxins and carcinogens. SNPs in GSTP1 have been associated with COPD [76], the distribution of emphysema [77] and more rapid decline in lung function [78]. However, the data should be interpreted with caution as the third of the cohorts [78] has been used in multiple analyses [69] and there was a lack of Hardy-Weinberg equilibrium for GSTP1 in their population suggesting either a systematic defect in genotyping or an unidentified bias in the selection of subjects. Moreover, no convincing association was found in other studies [71,79]. The null mutation of

GSTM1 (1p13.1) has also been associated with COPD [64], but others have failed to reproduce this finding [72].

Heme oxygenase catalyses the first step in heme degradation. Heme oxygenase 1 (HMOX1; 22q13.1) is the inducible isoform that can be upregulated by a wide range of stresses. Bile pigments generated by heme cleavage are believed to have antioxidant properties, thus HMOX1 induction is protective during cellular oxidant injury and over-expression of HMOX1 in lung tissue protects against hyperoxia. The HMOX1 gene 5'-flanking region contains stretches of GC repeats that are highly polymorphic in length. An early report found a higher proportion of long repeats in patients with COPD and also demonstrated that long repeats were associated with impaired promoter activity [80]. Attempts to reproduce this effect have had varied success [65,78,81,82]. While HMOX1 GC-repeat length has not convincingly been shown to be associated with developing COPD, there are some data to support an association between the long allele and increased severity of disease [82,83], although a recent study of smokers in the NHLBI Lung Health Study found no association between five HMOX1 SNPs and the decline of lung function [84]. Moreover, that study failed to detect evidence that the promoter polymorphisms affected regulation of the HMOX1 gene.

Superoxde dismutase (SOD) is an important antioxidant enzyme that catalyses the conversion of superoxide to oxygen and hydrogen peroxide. The extra-cellular isoform (SOD3; 4p15) is abundant in lung parenchyma. In the cross-sectional Copenhagen Heart Study, the R213G allele that results in higher plasma levels was associated with significantly less COPD in smokers [85]. A second study found similar results for the SOD3 isoenzyme, but not for other forms of SOD [86].

Whilst biologically very plausible, current genetic evidence fails to provide clear support for the involvement of detoxifying enzymes in the pathogenesis of COPD. Since the potential list of candidates to detoxify cigarette smoke remains long, it would be preferable if future studies were to take an unbiased approach to target identification rather than studying small numbers of candidate genes.

Inflammation

Tumour necrosis factor-α (TNF; 6p21) is a multifunctional cytokine whose levels are elevated in bronchoalveolar lavage, induced sputa and biopsies from patients with COPD. It is a plausible candidate gene for susceptibility to inflammatory disease, especially as well-studied promoter polymorphisms clearly alter expression levels. Consequently, considerable effort has been invested into determining whether the promoter polymorphism in TNFα also predisposes smokers to COPD. Much interest was generated when an early study revealed an association (with a staggering odds ratio of over 10) between allele 2 and 'bronchitis' in Taiwanese men [87]. This study is difficult to interpret as a third of the men were 'never smokers'. Despite some supportive evidence [88], many subsequent studies appeared to find little evidence that TNF polymorphisms are associated with, or modify the progression of COPD [69,74,89-98].

Group specific component (GC; 4q12), also known as vitamin D binding globulin, is a multifunctional protein that enhances the neutrophil and monocyte chemotactic activity of complement component 5a. It is a highly polymorphic protein with more than 124 forms, although three, Gc*1F, Gc*1S and Gc2, make up the majority. Kueppers and colleagues found Gc2 homozygotes to be protected from

COPD [99]. Others have seen this protective effect [100,101], while Gc*1F homozygosity has been found to be associated with COPD [102,103]. However, a much larger recent study has failed to reproduce these associations [104].

Conclusion

While environmental exposure to smoke remains the preeminent risk factor for developing COPD, the evidence that heredity plays a major role in an individual's risk is clear. The combination of GWAS and carefully conducted candidate gene approaches is helping to tease out those genetic variants responsible for the familial clustering of this disease, offering both the personalisation of individual risk stratification and, more excitingly, the hope for rational therapeutic interventions based on a better understanding of the underlying molecular pathology. The confusion surrounding many of the early (and some current studies) lies almost entirely with study power. Apart from the notable exception of SERPINA1, the contribution of individual genetic variants to risk of disease will prove to be small, for this reason large stratified cohorts of well-phenotyped individuals are likely to prove invaluable. A recent large systematic review of all case control candidate genetic studies in COPD prior to 2008 concluded that although the majority of such studies were underpowered to detect small genetic effects (OR 1.2-1.5), four genetic variants (or the 27 for which adequate data were available) remained significantly associated with COPD: the GSTM1 null variant (OR 1.45), rs1800470 in TGFB1 (0.73), rs1800629 in TNFα (OR 1.19) and rs1799896 in SOD3 (OR 1.97) [105]. Such findings, combined with the hypothesis generating observations from GWAS will direct COPD research for the next decade.

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Figure 1. **Extracellular matrix**. The extracellular matrix is comprised primarily of collagen and elastin fibrils. When mutated, many of the components of these fibrils and enzymes involved in their assembly leads to true connective tissue disorders that are associated with premature emphysema. Human disorders linked to mutation of specific components are labelled in bold; those for which murine models exist are then given in non-bold text.

Figure 2. **Candidate COPD genes at the alveolus**. Toxic compounds in smoke are inhaled to the alveolus where some are detoxified. Many candidate modifier genes of COPD encode enzymes mediating this detoxification. According to the current *protease – anti-protease* model of smoke-induced lung damage, toxins that escape these protective mechanisms inactivate anti-proteases enabling the degradation of extracellular matrix. Variants of genes encoding anti-proteases, proteases, components of the extra-cellular matrix and signalling pathways that regulate regeneration have all been implicated in COPD.