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Genetic Risk for Developing Chronic Obstructive Pulmonary Disease

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ABSTRACT

Chronic obstructive pulmonary disease (COPD), a disorder characterised by chronic airway inflammation and the development of emphysema, results from complex interaction between genetic and environmental factors, most importantly cigarette smoke. Linkage analysis, candidate gene studies, genome-wide association studies, and more recently exome and whole genome sequencing have identified numerous genes and variants linked to COPD susceptibility, smoking behaviour and determinants of lung function. This has led to insights into disease pathogenesis and has the potential to result in novel therapies for the condition. However, the genetic architecture of COPD is complex, and though many susceptibility loci have been identified, they only explain a fraction of disease heritability. Newer methodologies including exome sequencing and whole genome sequencing, and the study of epigenetic and gene-by-environment interactions may uncover some of this missing heritability. The focus of this review will be to assess the role of genetics in individual susceptibility to COPD. **(BRN Rev. 2019;5(3):154-68)**

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a heterogeneous condition characterised by chronic airway inflammation and the development of emphysema[1]. It affects approximately 174 million individuals worldwide[2], the majority of whom are smokers and it is the third leading cause of death in the USA[3]. The Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) defines COPD as "characterised by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases"[1]. The most important risk factor for the development of COPD is cigarette smoking[1], with COPD usually developing after smoking for an average of 20 to 30 pack-years. Environmental factors including pollution, wood smoke and occupational exposures also play a role in the development of COPD[1,4] and COPD, based on the spirometric definition of chronic airflow obstruction, can also be observed in asthma and conditions such as cystic fibrosis (CF). However, even among heavy smokers, only 15-20% develop COPD[5] and not all of those with COPD have a history of smoking[4], suggesting that there is individual susceptibility to environmental stress and/or factors independent of environment exposures including genetic susceptibility and gene-by-environment interactions that contribute to disease pathogenesis. Additionally, familial aggregation has been observed in COPD[6,7], and several monogenetic conditions including alpha-1 antitrypsin (AAT) deficiency and cutis laxa are associated with the development of COPD, further supporting a role for genetic susceptibility in disease pathogenesis.

Genetic linkage and candidate gene studies, genome-wide association studies (GWAS), exome and whole genome sequencing (WGS) have identified genes and loci associated with the condition[8,9]. Importantly, these studies have the potential to provide insights into COPD pathogenesis, improve risk prediction and lead to the development of new therapies for the disease and to the personalisation of COPD care[10]. However, though numerous COPD susceptibility loci have been identified[8], they only explain a fraction of disease hereditability[5,11-13].

The focus of this review will be to assess the role of genetics in individual susceptibility to COPD, including exploring the links between the condition and genetic variants associated with lung function parameters such as forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC), important quantitative traits given that COPD is defined based on an FEV_1 to FVC ratio of < 0.7[1]. Additionally, the genetic determinants of smoking behaviours and clinically relevant COPD phenotypes such as chronic bronchitis and emphysema will be examined.

SINGLE GENE HEREDITARY DISORDERS AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Alpha-1 antitrypsin deficiency, the most common hereditary disorder worldwide accounts for approximately 1 to 3% of COPD[14], including a small proportion of COPD in never smokers. It is a proven genetic risk factor for COPD, with the World Health Organization (WHO) recommending that all individuals diagnosed with COPD be screened once for AAT deficiency[1]. It is an autosomal recessive

Table 1. Alpha-1 antitrypsin deficiency modifier polymorphisms shared with non-alpha-1 antitrypsin deficiency chronic obstructive pulmonary disease susceptibility polymorphisms

Chr	Gene	SNP	AAT phenotype	AAT study cohort	References
Chr 7	NOS3	774T allele 894T allele	Airflow obstruction	PiZZ, controls	[8, 17]
15q25	CHRNA3 IREB2 LOC123688	rs1051730 rs2568494 rs8034191	Airflow obstruction	PiZZ, controls	[16]
Chr 11	GSTP1	105Val SNP	Airflow obstruction	PiZZ, controls	[8, 92]
Chr 6	TNF	rs361525 rs1800610 rs769178 rs3091257	Chronic bronchitis Airflow obstruction	PiZZ, controls	[8, 93, 94]
Chr 1	IL-10	rs1800871 (-819) rs1518110 rs1800896 (-1082) rs1878672 rs3024496 rs3024498	Airflow obstruction	PiZZ, controls	[94]

Gene polymorphisms which influence both the development and/or severity of lung disease in AAT deficiency, and COPD susceptibility.
AAT: alpha-1 antitrypsin; chr: chromosome; CHRNA3: cholinergic nicotine receptor alpha3; COPD: chronic obstructive pulmonary disease; GSTPI: glutathione s-transferase pi; IL: interleukin; IREB: iron regulatory binding protein; NOS: nitric oxide synthase; SNP: single nucleotide polymorphism; TNF: tumour necrosis factor.

condition resulting from the inheritance of two deficiency alleles in the SERPINA1 gene (usually severe deficiency occurring in Z allele homozygotes [i.e., PiZZ]) leading to a deficiency of AAT protein, an inhibitor of serine proteinases, primarily neutrophil elastase. This results in a protease/anti-protease imbalance in the lung which leads to the development of emphysema via elastin degradation and lung tissue destruction. Smokers with AAT deficiency typically develop emphysema about 10 to 20 years earlier than normal (i.e., PiMM genotype) smokers, however, there is significant heterogeneity in the development and severity of lung disease even in individuals with severe AAT deficiency. Additionally, PiZZ non-smokers can also develop COPD, but later in life, and with mild loss of lung function and generally their life expectancy is similar to that of never smokers[14,15]. These observations suggest that additional environmental factors and/or genetic modifiers influence susceptibility to lung disease in AAT. This observation is supported by familial aggregation studies and reports of associations among polymorphisms in nitric oxide synthase (NOS) 3, glutathione s-transferase pi (GSTPI), tumour necrosis factor (TNF), interleukin-(IL)10, iron regulatory binding protein (IREB)2 and cholinergic nicotine receptor alpha3 (CHRNA3) and COPD phenotypes including lung function in AAT deficiency, suggesting that there are other genes which act as modifier genes in the condition[16,17]. Many of these genes have also been linked to COPD susceptibility in normal individuals (Table 1)[8,9,18,19].

About 1 in 30 individuals in the USA are heterozygous for mutations associated with AAT deficiency, the vast majority of which are of the PiMZ genotype. There has been significant controversy over whether there is an association between PiMZ heterozygosity and an increased risk of COPD[20]. A meta-analysis of

16 case-control and cross-sectional studies with COPD as an outcome variable found a small association with increased risk of COPD in PiMZ heterozygotes versus PiMM controls, however there was no significant difference in mean FEV_1[21]. A subsequent study of two independent cohorts (total n=4,376) investigated differences between FEV_1, FEV_1/FVC ratio and high-resolution computed tomography (HRCT) measures of emphysema in PiMZ versus PiMM individuals, finding a lower FEV_1/FVC ratio in PiMZ heterozygotes, but no significant difference in FEV_1[22]. Additionally, differences in the extent of emphysema on HRCT were inconsistent between the two study populations, though a subgroup of PiMZ light smokers (< 20 pack-years) had more emphysema than PiMM controls in both cohorts. Analysis of the Genetic Epidemiology of COPD (COPDGene) study population (n= 8,271 non-Hispanic white and African American individuals, all ≥ 10 pack-year smokers) found that PiMZ heterozygotes had a lower FEV_1 and FEV_1/FVC ratio and more emphysema on HRCT than PiMM individuals[23]. Similarly, a family-based study found significantly lower FEV_1 and FEV_1/FVC ratio in PiMZ heterozygotes versus PiMM controls, which was linked to smoking status[24]. Taken together, these studies suggest a significant, but at most mild, relationship between the AAT MZ genotype and smoking status, with a small association between PiMZ heterozygosity and obstructive lung disease in smokers, with never smokers at no increased risk of COPD[14,20].

Cystic fibrosis is an autosomal recessive condition caused by mutations in cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-activated chloride and bicarbonate channel. The most important disease mutation is a deletion of phenylalanine at position 508 (F508del) though over 2,000 variants with a variety of effects on protein function have been described. These mutations lead to dehydration and acidification of airway surface liquid with increased mucus viscosity, impaired mucociliary clearance, chronic infection and airway inflammation resulting in progressive obstructive lung disease[25]. Individuals heterozygous for the F508del mutation in CFTR have a 50% reduction in CFTR expression, and this carrier status has been linked to an increased incidence of asthma and reduced lung function (FEV_1, FVC), although no increase in COPD prevalence has been reported[26]. However, other CFTR variants (R75Q, M470V) have been linked to the development of COPD[27]. Airway mucus plays important roles in pathogen killing and protection against xenobiotics including those found in cigarette smoke. Cystic fibrosis transmembrane conductance regulator has the ability to adapt to the environment, and through the regulation airway epithelial chloride and bicarbonate secretion can modulate airway mucus characteristics[27]. Though CFTR gene mutations have not been definitively linked to COPD susceptibility, acquired CFTR dysfunction has been suggested in smokers and COPD, and CFTR dysfunction correlates with COPD severity[28-31]. These observations suggest a role for CFTR in COPD pathogenesis and that it may represent a potential target for therapy.

Other monogenic disorders associated with COPD include the connective tissue disorders cutis laxa and Marfan's syndrome. Cutis laxa is a rare syndrome of loose, inelastic skin, and pulmonary and cardiovascular defects associated with heterozygous mutations in the elastin gene[32], linked to the development of emphysema in smokers and nonsmokers[33,34].

Marfan's syndrome results from a defect in the fibrillin-1 gene which leads to cardiovascular, skeletal, ocular abnormalities, spontaneous pneumothorax and emphysema[35]. Ehlers-Danlos syndrome is associated with the formation of pulmonary blebs, but does not lead to COPD[36]. Similarly, Birt-Hogg-Dube and familial spontaneous pneumothorax, both associated with mutations in the folliculin gene, also lead to pulmonary blebs with no clear association with COPD[4,37,38].

COMPLEX GENETICS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Familial aggregation studies of COPD and lung function, relevant as airflow obstruction is a central feature of COPD, have demonstrated an increased prevalence of COPD in relatives of COPD cases and significant heritability of lung function indices, providing evidence that genetic predisposition plays a role both in COPD susceptibility and in the determination of lung function[6,7,9].

The study of the genetics underlying the development of COPD began with linkage and candidate gene studies. As genotyping technologies advanced, this was followed by GWAS, which has the ability to test thousands of variants across the genome for association with a given trait. This technology revolutionised gene-association studies and led to the identification of many COPD and lung function susceptibility loci and genes[8,9,19]. Individual GWAS studies have subsequently been combined in meta-analyses, and this combined with improved imputation, a process which allows the confident prediction of unsequenced genotypes through the use of known population haplotypes, have expanded both the study population and single-nucleotide polymorphism (SNP) numbers available for testing, greatly increasing the power to detect disease-associated variants. Further advances in technology coupled with improved affordability have led to the use of exome and WGS to study the genetic basis of COPD. While GWAS identifies common variants (minor allele frequency [MAF] > 5%) with small effects, exome sequencing and WGS have the ability to identify rare variants with a large effect size. Many of the variants identified by GWAS are in noncoding regions of the genome. To understand the functional consequences of these variants, GWAS has been integrated with gene expression data from various sources including lung tissue, sputum and blood identifying expression quantitative trait loci (eQTL) linked to COPD. Susceptibility to COPD is also driven by epigenetic variation including changes in deoxyribonucleic acid (DNA) and histone methylation. Adding to the complexity of understanding COPD pathogenesis, DNA methylation is modified by environmental exposures such as cigarette smoke, and it is also subject to genetic control.

LINKAGE AND CANDIDATE GENE STUDIES

Linkage analysis, which uses family data from multiple generations to study haplotypes or genes and sequences inherited together due to their chromosomal location, has been used to investigate genes that co-associate with disease. This approach has led to the identification of large genomic regions on many chromosomes including 1, 2, 4, 6, 12, 18, 19, 21 and 22 linked with lung function indices and/or COPD[39-41]. For example, using families of

probands with severe early-onset COPD, linkages were observed between lung function parameters including mild (chromosomes 8, 19) and moderate (chromosomes 12, 19) airflow obstruction, and chronic bronchitis (chromosomes 19, 22), with stronger linkage observed in certain regions in smoker-only analyses[40]. Similarly, a genome-wide linkage analysis study of bronchodilator responsiveness phenotypes, an important component of COPD, identified an association with loci located on chromosomes 1, 2, 3, 4 and 8 in families of probands with early-onset COPD[42]. Interestingly, several of the chromosomal regions identified in these studies overlap with regions identified in prior asthma genome linkage analyses[42].

Candidate gene studies are hypothesis-driven, investigating polymorphisms in genes with potential roles in disease pathogenesis based on their known biologic functions. In COPD this approach has led to the identification of more than 100 candidate genes. There are significant limitations associated with many of these studies including inadequate power and variability in the definition of COPD. It has not been possible to replicate many of the candidate gene findings, making it difficult to draw definitive conclusions regarding the association between many of these genes and COPD susceptibility (Table 2)[5,8,43,44]. Despite these caveats, a meta-analysis of 20 polymorphisms in 12 genes identified variants in transforming growth factor (TGF)-β1, interleukin 1 receptor antagonist (IL1RN), TNF-α and GSTP1 which were associated with COPD, with the magnitude of many of these associations influenced by ethnicity[43]. Another meta-analysis of candidate gene studies up to 2009 found variants in four genes - GSTM1 (null variant), TGF-β1 (rs1800470), TNF-α (rs1800629) and superoxide dismutase (SOD)3 (rs1799896) - were significantly associated with COPD with odds ratios ranging from 0.73-1.97[44].

Table 2. Top chronic obstructive pulmonary disease candidate genes and associated biological pathways

Gene	Biologic pathway	Reference
EPHX1	Xenobiotic metabolic process	[8, 9]
SERPINA1	Anti-protease	[8, 9]
GSTP1	Xenobiotic metabolic process, redox	[8, 9, 43]
TNF	Cytokine	[8, 9, 44]
GSTM1	Xenobiotic metabolic process, redox	[8, 9, 44]
TGF-β1	Cytokine	[8, 9, 43, 44]
ADR-β2	β2-adrenergic receptor activity	[8, 9]
SOD3	Anti-oxidant	[8, 9, 44]
ACE	Peptidase activity	[8, 9]
ADAM33	Peptidase activity	[8, 9]
IL-1β	Cytokine	[8, 9]
IL-6	Cytokine	[8, 9]
CFTR	Chloride channel	[8, 9, 27]
HMOX1	Heme catabolism	[8, 9]
IL-10	Cytokine	[8, 9]
SERPINA3	Anti-protease	[8, 9]
SFTPB	Surfactant protein	[8, 9]
GC	Vitamin D binding protein	[8, 9]
MMP9	Protease	[8, 9]
SERPINE2	Serine protease inhibitor	[8, 9]
GSTT1	Xenobiotic metabolic process, redox	[8, 9]
IL-13	Cytokine	[8, 9]

Top genes based on multiple publications supporting an association with COPD[8,9].
CFTR: cystic fibrosis transmembrane conductance regulator; COPD: chronic obstructive pulmonary disease ; MMP: matrix metalloproteinase. For other abbreviations see table 1.

GENOME-WIDE ASSOCIATION STUDIES AND META-ANALYSES

Since the first GWAS of lung function in 2007[45], the understanding of the genetics determining

lung function indices and COPD susceptibility has increased exponentially with the identification of many loci associated with COPD and/or lung function at a stringent statistical cut-off of $p \leq 5\times10^{-8}$. This initial GWAS included over 1,000 individuals participating in the Framingham Heart Study. Although no results met the strict criteria for genome-wide significance, this study identified GSTO2 and IL6R as lung function candidate genes[45]. This was followed by a larger study (n=7,691) which reported an association between FEV_1/FVC ratio and SNPs in 4q31 region close to the hedgehog-interacting protein (HHIP) gene[46]. An accompanying manuscript, the first COPD GWAS[47], identified the 4q31region as a COPD susceptibility locus in addition to SNPs in α-nicotinic acetylcholine receptor (CHRNA3/CHRNA5) in the 15q25 locus, a region previously associated with lung cancer risk[48]. Subsequently, GWAS have identified associations between the 4q22.1 (family with sequence similarity 13 member A [FAM13A]) and 19q13 (RAB4B, EGLN2, MIA, CYP2A6) chromosomal regions and COPD[49,50]. Interestingly, 19q13 has also been associated with cigarette smoking behaviour[51,52], lung function and emphysema distribution in GWAS, while transcriptome analysis has demonstrated that smoking dysregulates the expression of several genes linked to COPD at this locus in airway stem/progenitor cells[53]. Further GWAS and meta-analyses have uncovered novel COPD risk loci including at RIN3, MMP12 and TGF-β2 in addition to replicating known chromosomal loci 15q25 (CHRNA3/CHRNA5/IREB2), 19q13, and regions near the genes HHIP and FAM13A[54]. For a comprehensive review, see table 3. Although these loci have been replicated convincingly in large populations, overall they explain only a small fraction of COPD risk[8.9.19.44].

Table 3. Definite chronic obstructive pulmonary disease susceptibility loci identified in genome-wide association studies[1,2]

Locus	Gene*	SNP	Reference
1q41	TGF-β2	rs4846480	[54]
4q22	FAM13A	rs1903003 rs7671167 rs4416442 rs1964516	[49, 50, 54]
4q31	HHIP	rs1828591 rs13118928 rs13141641	[47, 49, 54]
11q22	MMP12	rs626750	[54]
14q32	RIN3	rs754388	[54]
15q25	CHRNA3	rs8034191 rs1062980	[47] [49]
	CHRNA5	rs1051730 rs17486278	[47, 49, 54, 58]
	IREB2	rs13180	[49]
19q13	RAB4B/EGLN2	rs7937	[50]
	RAB4B/EGLN2	rs2604894	

*Gene for which the associated SNP is within or closest to if located in a noncoding region.
Definite COPD susceptibility loci based large GWAS/meta-analyses supporting an association with COPD[8,9,19].
SNP: single nucleotide polymorphism. For other abbreviations see tables 1 and 2.

Since the first GWAS study of genetic determinants of lung function identified variants in 4q31 near HHIP[46], over 30 common genetic variants (MAF > 5%) associated with spirometric measures of lung function (FEV_1, FEV_1/FVC ratio) have been identified. These studies and meta-analyses have included large subject and genetic marker numbers including nonsmokers, smokers and individuals with COPD. For example, a recent GWAS included n=48,943 in the discovery and n=95,375 individuals in the follow-up cohort, identifying 97 lung function signals including 43 novel variants and generating a genetic risk score associated with COPD susceptibility[55]. Unsurprisingly, given that COPD is defined based on lung function parameters, there is significant

overlap between these loci and variants and those associated with COPD status including at 4q31 (HHIP), 4q22.1 (FAM13A) and 15q25[8.9.19,41,45,46,55.57]. For a detailed review of overlapping lung function and COPD loci, see table 4. Additionally, overlap has been reported in variants associated with COPD, lung function, and pulmonary fibrosis including two shared loci (FAM13A and desmoplakin [DSP]) with the opposite risk alleles in COPD and pulmonary fibrosis[58]. Smoking is a common risk factor for COPD and lung cancer, of the lung cancer loci identified to date, many including 15q25 and FAM13A overlap with COPD susceptibility loci[48,59,60]. Finally, GWAS has identified 2 novel regions on chromosomes 10 and 14 associated with lung function decline in COPD[61].

There is significant phenotypic heterogeneity in COPD including clinical (chronic bronchitis, exacerbations, the asthma-COPD overlap syndrome [ACOS]) and radiological (presence and distribution of emphysema) phenotypes[1]. GWAS have elucidated some of the genetic variants that may contribute to this phenotypic variation. Chronic bronchitis, a major COPD phenotype, has been linked to variants in 11p15 (EFCAB4A, CHID1, AP2A2) in addition to the previously described COPD locus at 4q22 (FAM13A)[62]. Mucus hypersecretion, a frequent symptom in COPD has been associated with rs6577641, a variant in SATB1[63]. The ACOS, defined as "persistent airflow limitation with several features usually associated with asthma and several usually associated with COPD"[64], has been linked by GWAS to variants in CSMD1, SOX5 and GPR65[13]. Emphysema is associated with variants in BICD1[65], while HRCT-identified emphysema distribution, as measured by the difference between extent of emphysema upper-third and lower-thirds of the lung on HRCT and the ratio of upper-third to lower-third emphysema, has been linked to variants in the known COPD loci 4q31 (near HHIP) and 15q25 (near CHRNA5) and to novel loci near SOWAHB, TRAPPC9, and KIAA1462[66]. Additionally, many previously described COPD loci are associated with COPD phenotypes including pack-years smoked (CHRNA3/5), the presence of emphysema on HRCT chest imaging (CHRNA3/CHRNA5, HHIP), lung function (FEV_1/FVC ratio) (CHRNA3/CHRNA5, HHIP), FEV_1 (CHRNA3/CHRNA5, IREB2, FAM13A), body fat (HHIP) and exacerbations (HHIP)[67].

The GWAS studies have focused on genetic susceptibility to various clinical traits in COPD. These include identifying associations between body mass index and fat-free mass index and alpha-ketoglutarate dependent dioxygenase (FTO) in individuals with COPD[68]; and between two loci on chromosomes 14 and 15 and resting oxygen saturation in African Americans with COPD and several suggestive loci in individuals of European descent with COPD[69]. Other associations induced variants near IREB2 and GALC associated with pulmonary artery enlargement on HRCT (a correlate of pulmonary hypertension) in individuals with COPD[70]. Additionally, a GWAS has linked response to inhaled β_2-agonists in COPD to variants in KCNK1, KCNJ2 and CDH13[71]. The GWAS have also investigated genetic determinants of circulating biomarkers levels in COPD, finding an association between two loci near the SCGB1A1 gene and club cell secretory protein and with multiple SNPs on chromosomes 6 and 16 and near SFTPD and surfactant protein D. However, no variants have been linked to levels of the systemic inflammatory markers fibrinogen, IL-6, IL-8,

Table 4. Top single nucleotide polymorphisms associated with both chronic obstructive pulmonary disease susceptibility and lung function parameters in genome-wide association studies

Gene	SNP	Lung function phenotype	Lung function study cohort (n)	Reference
ADAM19	rs2277027	FEV_1/FVC ratio	16,178	[95]
NCR3	rs2857595	FEV_1/FVC ratio	46,411	[96]
HHIP	rs1980057 rs12504628 rs13147758 rs13141641	FEV_1/FVC ratio FEV_1/FVC ratio, FEV_1 (litres) FEV_1/FVC ratio FEV_1/FVC ratio	16,178 19,400 835 835	[46, 58, 95, 97]
TGF-β2	rs10429950	DLCO % predicted	6,935	[54, 58, 98]
AGER	rs2070600	FEV_1/FVC ratio	16,178	[95, 97]
ARMC2	rs2798641	FEV_1/FVC ratio	46,411	[96]
TET2	rs2047409	FEV_1 extreme (low)[1]	50,008	[58, 75]
CDC123	rs7068966	FEV_1/FVC ratio	46,411	[96]
HDAC4	rs12477314	FEV_1/FVC ratio	46,411	[96]
CCDC38	rs1036429	FEV_1/FVC ratio	46,411	[96]
C10orf11	rs11001819	FEV_1/FVC ratio	46,411	[96]
THSD4	rs12899618	FEV_1/FVC ratio	19,875[2]	[97]
KCNE2	rs9978142	FEV_1/FVC ratio	46,411	[96]
NPNT	rs17331332	FEV_1/FVC ratio, FEV_1 (litres)	20,890	[95]
TNS1	rs2571445	FEV_1/FVC ratio, FEV_1 (litres)	18,710[2]	[97]
RARB	rs1529672	FEV_1/FVC ratio	46,411	[96]
CFDP1	rs2865531	FEV_1/FVC ratio	46,411	[96]
GPR126	rs381792	FEV_1/FVC ratio	16,178	[38, 95]
FAM13A	rs2869967	FEV_1/FVC ratio	16,178	[49, 58, 95]
HTR4	rs3995090 rs11168048	FEV_1/FVC ratio FEV_1/FVC ratio	16,178; 18,792[2] 20,890	[58, 95, 97, 99]
ZKSCAN3	rs6903823	FEV_1/FVC ratio	46,411	[96]
MFAP2	rs2284747	FEV_1/FVC ratio	46,411	[96]
GSTCD	rs10516526	FEV_1 (litres), FVC (litres)	16,178; 20,179[2]	[95, 97]

[1] FEV_1 extreme-study cohort were divided into 3 groups based on FEV_1% predicted distribution (low, average, high).
[2] Effective sample size- product of sample size and imputation quality metric summed across studies to calculate total effective meta-analysis sample size[97].
Top SNPs based large GWAS/meta-analyses findings[8,9,19].
COPD: chronic obstructive pulmonary disease; DL_{CO}: diffusing capacity of the lung for carbon monoxide; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; SNP: single nucleotide polymorphism. For further abbreviations see tables 1 and 2.

TNF-α, and C-reactive[72]. Similarly, protein quantitative trait loci (pQTL) analysis was used to identify SNPs associated with a panel of 88 blood biomarkers, with predominantly novel pQTL identified in 43% of the proteins tested that explained > 10% of the measured variation in 13 protein biomarkers[73].

Cigarette smoking is the single most important modifiable risk factor for the development

of COPD[1]. Smoking behaviour, including smoking quantity (cigarettes smoked per day [CPD]) and nicotine dependence, are recognised to have environmental and genetic influences, some of which overlap with COPD. Large GWAS studies of smoking behaviour have identified several associated loci, with the strongest association with variants in 15q25 locus which contains genes encoding the nicotinic receptor subunits CHRNA3-CHRNA5-CHRNB4, a region previously linked to COPD and lung cancer, in addition to the COPD locus 19q13 and novel regions including 8p11, 9q13 and 10q25 with CPD[51,52,74]. Other novel genetic variants have been associated with smoking behaviour including a variant in NCAM1 (chromosome 11) and a variant on chromosome 2 between TEX41 and PABPC1P2[75]. Another study, combining GWAS of common variants and exome sequencing, identified an association between 15q25 and CPD, although exome sequencing analysis of a subgroup of subjects did not yield any significant rare variants[76]. Finally, in addition to replication of the association between CPD and the CHRNA3/CHRNA5 and CYP2A6 loci, GWAS also identified a link between variants in DBH and smoking cessation[77].

EXOME AND WHOLE GENOME SEQUENCING

In contrast to GWAS which identifies common variants with small effects, exome sequencing in which the protein-coding region of the genome (approximately 1.5%) is sequenced, has the potential to identify rare variants with large effects. To date, several COPD exome sequencing studies have been performed which have both identified novel COPD-related SNPs and confirmed loci identified in previous GWAS. These include novel COPD-associated SNPs in MOCS3 and IFIT3, and a splice variant in SERPINA12 linked to airflow limitation (FEV_1) and the previously reported loci in MMP12, HHIP, GPR126 and CHRNA5[78]; and a novel SNP in IL27, and in addition to known SNPs in CHRNA5, AGER, MMP3, SERPINA1[79]. For a comprehensive list of COPD genes and SNPs identified by exome sequencing, see table 5.

Exome sequencing performed on 347 individuals from 49 pedigrees with severe early-onset COPD enrolled in the family-based Boston Early-Onset COPD Study identified 69 genes with predicted deleterious mutations that segregated with severe COPD in at least two families. Subsequent testing of these genes in resistant smoker versus individuals with severe COPD in the COPDGene study identified an increase in rare mutations in COPD in four of these genes (DNAH8, ALCAM, RARS, GBF1)[80]. In a follow-up study of severe COPD combining three study cohorts including the family-based Boston Early-Onset COPD Study and International COPD Genetics Network studies, and the case-control COPDGene study (total n=2,543) identified rare loss of function variants in TBC1D10A and RFPL1 in the combined familial studies using gene-based segregation testing. However, these variants were not replicated in the COPDGene cohort[81]. Despite this, analysis of the top variants from both study types suggested that they related to similar biological processes.

In addition to COPD, exome sequencing studies have focused on related traits including lung function, sensitivity to cigarette smoke, emphysema and lung cancer. A meta-analysis

Table 5. Genes associated with chronic obstructive pulmonary disease, lung function parameters and related phenotypes identified by exome and whole-genome sequencing studies

Study type	Gene	Phenotype	Reference
Exome sequencing	MOCS	Risk of COPD	[78]
	IFIT3	Risk of COPD	[78]
	SERPINA12	FEV_1% predicted	[78]
	MMP12	Risk of COPD	[78]
	HHIP	Risk of COPD	[78]
	GPR126	Risk of COPD	[78]
	CHRNA5	Risk of COPD	[78, 79]
	IL27	COPD	[79]
	AGER	COPD	[79]
	MMP3	COPD	[79]
	SERPINA1	COPD	[79]
	DNAH8	COPD	[80]
	ALCAM	COPD	[80]
	RARS	COPD	[80]
	GBF1	COPD	[80]
	RPAP1	FEV_1/FVC ratio	[82]
	SEC24C	FEV_1 (litres)	[82]
	CASC17	FEV_1 (litres)	[82]
	UQCC	FVC (litres)	[82]
	LY86	FEV_1/FVC ratio	[82]
	FGF10	FVC (litres)	[82]
	CCDC38	Resistance to smoking	[83]
	TACC2	Susceptibility to smoking	[84]
	MYO1E	Susceptibility to smoking	[84]
Whole-genome sequencing	ZNF816	Susceptibility to emphysema	[85]
	PTPRO	Susceptibility to emphysema	[85]
	HHIP	Risk of severe COPD	[86]
	SERPINA1	Risk of severe COPD	[86]

COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity. For other abbreviations see tables 1 and 2.

of exome array data and lung function parameters (FEV_1, FVC, FEV_1/FVC ratio), including n=68,470 individuals in the discovery and n=111,556 in the replication cohorts, identified six novel SNPs in RPAP1, SEC24C, CASC17, UQCC and two intergenic SNPs near LY86 and FGF10 linked to lung function, with eQTL analysis implicating TYRO3 and PLAU in lung function determination[82]. An exome sequencing study examining resistance to the detrimental effects of the cigarette smoke, identified a variant in CCDC38, previously associated with FEV_1/FVC ratio, and cilia-related pathways in heavy smokers (n=100, > 20 pack-years) with normal lung function[83]. Another case-control study, integrating exome sequencing and airway gene expression of sensitive (n=62) and resistant smokers (n=30), identified two candidate genes (TACC2 and MYO1E) which, when silenced in in vitro studies, augmented cigarette smoke toxicity[84]. To investigate the genetic factors underlying cigarette smoking, GWAS and exome sequencing analysis were combined to examine common and rare variants, respectively. Genome-wide significant variant associations with cigarettes per day were identified on chromosome 15q25 in the GWAS analysis. However, for the exome analysis in a smaller cohort, no variants reached significance[76].

Compared to exome sequencing, WGS provides coverage of the entire genome including non-coding regions and allows improved identification of variants in coding regions. To date, WGS performed in individuals with severe airflow obstruction resistant or susceptible to emphysema identified several suggestive candidate genes associated with emphysema with severe airflow obstruction. These included ZNF816 and PTPRO, a novel rare

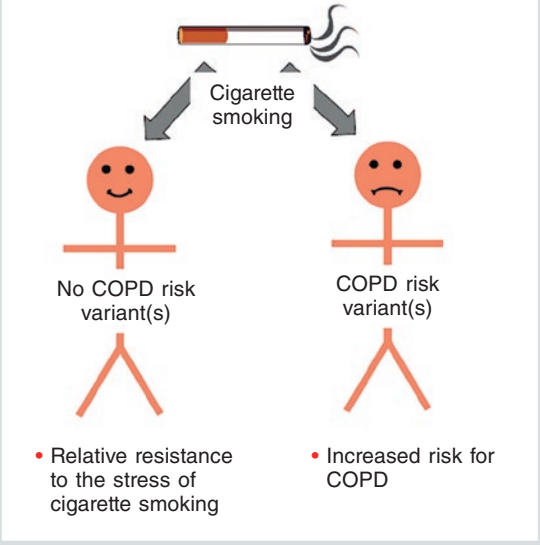

Figure 1. Chronic obstructive pulmonary disease (COPD) results from environmental exposures, most commonly cigarette smoking, in susceptible individuals with a genetic predisposition with variants associated with the development of COPD.

variant in PTPRO (rs61754411) contributing to emphysema susceptibility[85]. In a WGS study of the Boston Early-Onset COPD Study and COPDGene severe COPD cohorts (n=1,794), the strongest associations were observed in known COPD risk loci, including HHIP and SERPINA1[86].

INTEGRATIVE GENOMICS AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Most of the variants identified by GWAS are noncoding, with unclear function. Integrative approaches combining GWAS studies with gene expression data from disease-relevant tissues including blood, sputum and lung tissue to identify COPD eQTL have been utilized to gain insight into the target genes and biological relevance of genetic variants identified by GWAS. Expression quantitative trait loci are genetic loci that explain a portion of the tissue expression of one or more genes, linking genetic variants with expression of specific genes in disease-relevant tissues and helping to understand the mechanisms responsible for disease risk conferred by the genetic variant(s). For example, eQTL analysis of lung tissue gene expression and COPD susceptibility loci identified associations between the SNPs rs182859 and rs13118928 in 4q31 and HHIP gene expression and between SNPs in 19q13 and EGLN2 messenger (m)RNA levels[87]. Integration of COPD GWAS loci with whole blood and sputum gene expression identified 19 eQTLs associated with COPD and nearby gene expression including the previously identified loci near HHIP, FAM13A, and the 15q25 and 19q13 loci[88]. Additionally, in airway stem/progenitor cells cigarette smoking leads to changes in the expression of genes at the 19q13 locus including NFκBIB, LTBP4, EGLN2 and TGF-β1[53]. These studies support a key role for genetic control of gene expression in the genetic architecture of COPD.

EPIGENETICS AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Given that many of the COPD susceptibility variants identified by GWAS are in noncoding intergenic and intronic regulatory regions of the genome suggests that epigenetic variation may play a role in COPD susceptibility and pathogenesis. Epigenetic modifications including DNA and histone methylation and histone acetylation, which alter gene expression without a change in the DNA sequence,

are under both environmental, including nicotine-related[89], and genetic control. Understanding the relationship between gene polymorphisms and the epigenetic regulation in COPD may explain some of the "missing heritability" in the condition. A study comparing differentially methylated loci in lung tissue obtained from former smokers with COPD versus normal ex-smokers, integrated with previous GWAS results, identified top sites in CHRM1, GLT1D1, and C10orf11, with enrichment for known asthma and lung function genes and for transcription factors[90]. Similar to the integrative genomics approaches discussed above, methylation profiling has been integrated with SNP variant data to identify DNA methylation quantitative trait loci (mQTL). Using the same cohort as in the previous study, mQTL analysis of the DNA methylation profile of lung tissue obtained from ex-smokers with and without COPD was performed testing for SNPs associated with the degree of DNA methylation revealing colocalization of mQTL and COPD GWAS loci, supporting a role for genetic control of methylation in the pathogenesis of COPD[91].

CONCLUSIONS

Chronic obstructive pulmonary disease is a common condition with complex genetic architecture which results from complex interactions between environmental exposures, genetic susceptibility and gene-by-environment interactions. Genetic variation clearly plays a role in COPD susceptibility, but the genetic risk is small and very complex. Exome, WGS, epigenetic and integrative approaches have the potential to uncover a more complete picture of COPD hereditability.

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DISCLOSURES

Dr. Crystal and Dr. O'Beirne have nothing to disclose.

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