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Developmental genetics of the COPD lung

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Abstract

Chronic Obstructive Pulmonary Disease (COPD) is a debilitating disease of the lung which results in irreversible airflow obstruction and is currently the third leading cause of death worldwide. Genetic and environmental factors contributing to COPD are presently under investigation. As lung function measures cluster within families, we now know that lung function is partly inherited. Thus, identifying genes involved in determining lung function at the population level and in determining the risk of development of COPD is important. A thorough understanding of the mechanisms underlying maintenance of lung function and knowledge of how these are altered in lung disease could ultimately lead to targeted therapeutic approaches. This is of potential value in COPD because current treatments are designed to reduce symptoms but do not modify disease progression. Here, we review the genes identified from both meta-analyses of genome-wide association (GWA) studies of lung function in large populations and case control GWA studies in COPD. We hypothesise that mechanisms involved in the early development of the lungs may vary/alter and predispose to COPD later in life. We discuss the genes and pathways involved in normal lung development and ascertain whether they overlap with key genes identified from GWA studies. Epigenetic factors may also play an important role in lung function, development and disease. Furthermore, we discuss our findings on the functional characterisation of *HTR4* and genes within the 4q24 locus associated with both lung function and COPD. Lastly, we consider new genetic techniques and models to study candidate genes identified by the approaches discussed.

Keywords: COPD, Lung development, Lung function, Genetics, Heritability, Environment, FEV_1, FEV_1/FVC, Developmental signalling pathways

Introduction

Diseases which cause a decline in lung function remain a huge burden to human society and the economy. One such disease, Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous and debilitating condition characterised by the development of irreversible airflow obstruction. The development of COPD has a strong environmental basis, with cigarette smoking and exposure to poor air quality being key risk factors. Unlike some common chronic diseases, the incidence of COPD has not declined in recent years, in fact there continues to be increasing prevalence, morbidity and mortality rates for COPD globally. According to the World Health Organisation, 64 million people worldwide have COPD and > 3 million people die each year of the disease [1]. Within the UK alone it is estimated that 3 million people have COPD and it accounts for 30,000 deaths each year [2]. Critically, COPD is now the third leading cause of death worldwide, Fig. 1 [3].

In general, COPD is a progressive condition, leading to airway remodelling, inflammation and narrowing of the small airways and/ or alveolar destruction (emphysema), with symptoms generally becoming evident later in life [4]. Although the introduction of smoking bans may help to lower the incidence of COPD in some countries, not all patients with COPD are smokers [5]. It is also important to note that COPD can be caused by biomass exposure. However, in addition to environmental exposures, around 40 % of variability in lung function is estimated to be heritable [6–9]. There are a range of therapeutic agents available for treatment of COPD, including short and long acting β2 agonists, anti-muscarinic agents, inhaled and oral steroids and phosphodiesterase inhibitors: however, whilst these drugs can improve symptoms in some patients none of them have been show to alter the progression of underlying disease.

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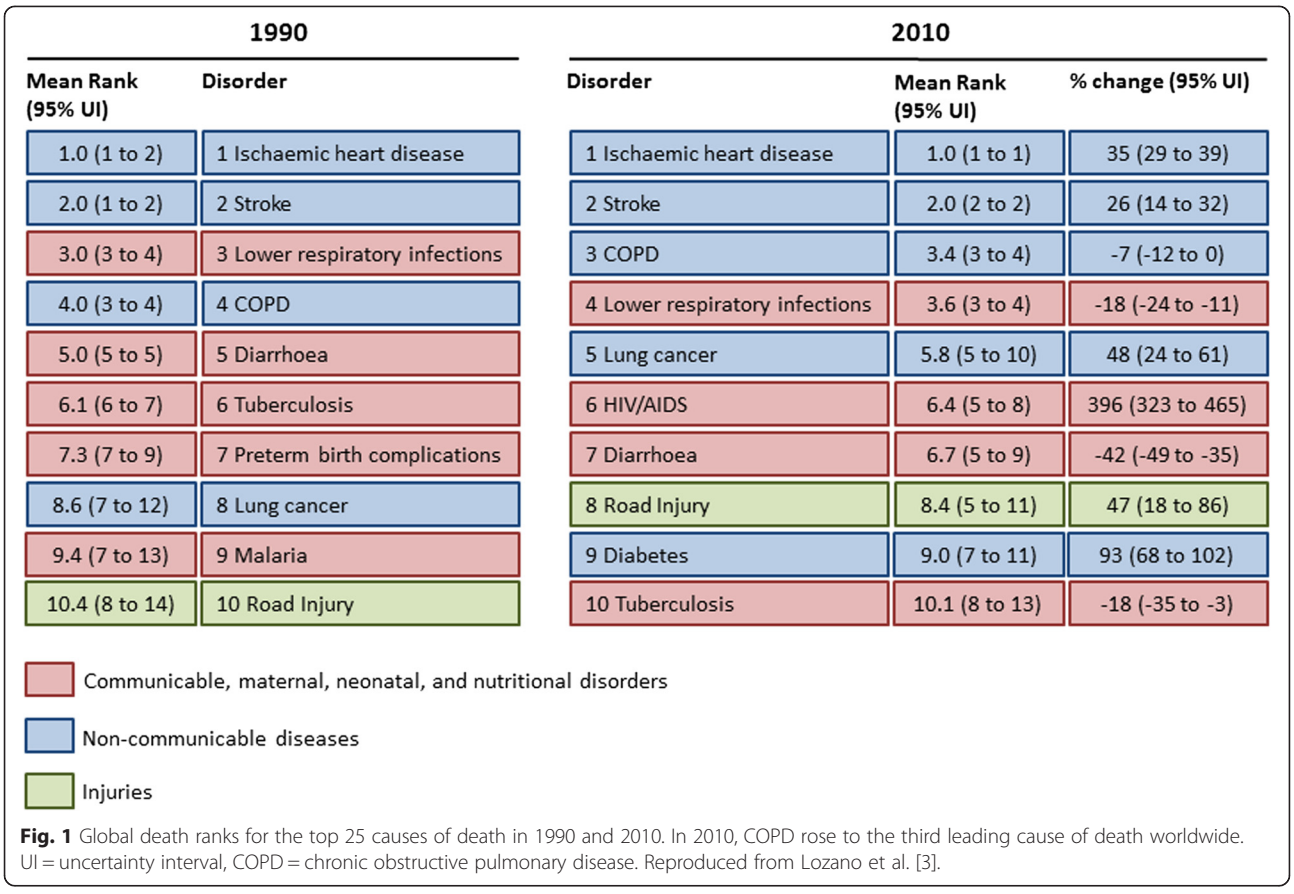

Fig. 1 Global death ranks for the top 25 causes of death in 1990 and 2010. In 2010, COPD rose to the third leading cause of death worldwide. UI = uncertainty interval, COPD = chronic obstructive pulmonary disease. Reproduced from Lozano et al. [3].

Review

Diagnosing COPD using spirometry

Spirometry is used to assess lung function in humans. The most useful measures are FEV_1 (forced expiratory volume in 1 s) and FVC (forced vital capacity, i.e., the volume of air expired by a full expiration). When the ratio of FEV_1 to FVC is under 0.7, this is referred to as an obstructive defect. The severity of COPD can also be assessed by spirometry, a value of FEV_1 less than 80 % predicted indicating (in the presence of a reduced FEV_1/FVC ratio) the presence of COPD. Interestingly, it has been shown that spirometry measurements also cluster within families again suggesting there is a hereditary component which may influence the development of respiratory disease [10, 11]. Between 20-60 % of phenotypic variance in lung function measures is suggested to be attributed by hereditary factors [6–9] and this is strongly correlated in twin studies [12].

Environmental and genetic factors of COPD

Smokers are characteristically prone to developing COPD; therefore smoking is a primary risk factor for developing COPD. Estimates indicate that after 25 years of smoking 30-40 % of smokers will have COPD [13]. Even non-smokers may be affected due to general exposure to air pollutants. One investigation into long term smoke particulate matter exposure revealed a significant association between an increase in exposure to small particles and a mild decrease in FEV_1 across 20 years [14]. In addition, biomass emissions are also a notable risk factor globally, in general consisting of smoke inhalation via indoor pollution or occupational exposure. Genetic predisposition is also a known risk factor which increases an individual's susceptibility to developing COPD. The most commonly studied example in COPD is α1-antitrypsin deficiency where individuals (commonly of northern European ancestry) are homozygous for a deleterious mutation in *SERPINA1* [15]. 1-2 % of COPD cases are attributable to this mutation, which leads to enhanced neutrophil elastase activity, ultimately leading to destruction of the alveoli. Early genetic linkage analyses have indicated the existence of gene-by-smoking interactions as contributing to a decline in lung function. In those studies the logarithm of odds (LOD) score of genetic linkage was improved by restricting the analysis to smokers which suggested the existence of interaction between cigarette smoke exposure and genetic susceptibility [16]. More recently Liao et al. have more robustly explored the effects of gene-by-environment interaction by using individual SNPs and genetic network approaches [17]. Both ways of analysis

identified SNPs near gene *SLC38A8* as significantly modifying the effects of occupational exposure on FEV_1. Genetic network analysis alone identified genes *CTLA-4*, *HDAC*, and *PPAR-alpha* as modulating these effects. This study implied the existence of genes related to inflammatory processes which could modify the effects of occupational exposure on lung function. Readers are advised to refer to an excellent review by Molfino and Coyle which reviews the gene-environment interaction in COPD [18].

Meta-analyses of GWA studies identifies genetic regions associated with FEV_1

Large scale genetic studies (genome wide association studies (GWAS)) are now able to accurately reveal associations between phenotypes (such as spirometry measures) and genetic loci. By meta-analysing many GWA studies, researchers have revealed a number of single nucleotide polymorphisms (SNPs) within/near genes which are associated with the lung function measure FEV_1 (Table 1). These genes may potentially influence the development or severity of COPD and could also be important in other obstructive diseases of the lung [19, 20].

Five meta-analyses and one look up of candidate SNPs identified from the SpiroMeta general population were included in the overview of GWAS meta-analyses in Table 1. In 2010, back to back publications by our group [19] and others [20] showed the utility of meta-analysing GWA studies when both studies identified SNPs within the 4q24 locus to be the most significantly associated with FEV_1. Hancock et al. identified 46 SNPs at this locus with the smallest p value for SNP rs17331332 located nearest *NPNT*, whilst the top SNP of our study is located in oppositely transcribed genes *INTS12* and *GSTCD* [20, 21]. Interestingly, a look up of previously suggested candidate genes found no significant associations suggesting that genome wide approaches are the most reliable way to identify true genetic risk factors for COPD and/or lung function phenotypes [22]. In the same year Soler-Artigas et al. reported 16 novel loci associated with lung function; 5 associated with FEV_1, 4 of which survived joint meta-analysis of all stages (*MECOM* (also known as *EVI1*), *ZKSCAN3*, *CDC123*, *C10orf11*) [23]. Subsequently in 2012, Hancock et al. identified *KCNJ2/SOX9* at 17q24.3 to be associated with FEV_1 [24]. Given that cigarette smoking adversely affects pulmonary function, the group conducted genome-wide joint meta-analyses of SNPs and SNP by smoking associations. GWAS have also been utilised to identify variants associated with smoking behaviour. In 2010, three pivotal publications identified loci associated with smoking behaviour. Whilst Thorgeirsson et al. identified variants in neuronal acetylcholine receptors, *CHRNB3-CHRNA6* and the Cytochrome P450, *CYP2A6* associated with smoking behaviour [25], Liu et al. refined the association identified at 15q25 [26]. In the same year the Tobacco and Genetics Consortium identified multiple loci associated with smoking behaviour [27].

More recently in 2014, Tang et al. studied longitudinal changes in lung function and mean rates of decline by smoking pattern. The strongest association with decline in FEV_1 mapped to SNPs at 15q25.1 encompassing *IL16/STARD5/TMC3*, however, this result did not reach genome-wide significance [28]. Furthermore, Tang et al. studied rate of FEV_1 change in a subsequent meta-analyses of 5 cohorts which had more than 3 measurements per participant. Interestingly, a SNP within *BAZ2B* was identified at both stages [28].

COPD associated genes

In addition to the study of the genetic basis for lung function in large populations, sixteen case control studies of COPD have also been studied to try and identify SNPs in genes which are associated with COPD (Table 2). In GWA studies of COPD cohorts, SNP rs7671167, within *FAM13A,* was associated with chronic bronchitis, airway obstruction, emphysema and COPD susceptibility [29–32]. Additionally 9 other SNPs within *FAM13A* were associated with COPD [29, 31–34]. This region is close to but distinct from the 4q24 locus identified by earlier studies on FEV_1 and FEV_1/FVC ratio. *HTR4* (encoding a serotonin receptor) was also found to be associated with COPD in two separate GWA studies [35, 36]. The 4q24 locus and *HTR4* are discussed in more detail in a later section.

In particular, 6 studies have found numerous SNP's at the 15q25.1 locus to be associated with COPD [30, 31, 34, 35, 37, 38]. This locus encompasses 3 cholinergic nicotinic receptors (*CHRNA5*, *CHRNA3* and *CHRNAB4*). However, this locus appears to exert its effects by determining an individual's risk for nicotine dependence rather than through any direct effect on the lung *per se*.

Current efforts within the respiratory research community are trying to decipher the biological relevance of the functions of these genes and elucidate whether pathways identified are therapeutically targetable. On comparison of the genes shown in Tables 1 and 2 presenting the top genes associated with either FEV_1 (from meta-analysing GWA studies) or COPD, the most obvious priorities for further research would appear to include *TNS1*, genes within the 4q24 locus (*FLJ20184, INTS12, GSTCD* and *NPNT*), *HHIP, HTR4* and *SOX9*. Within the genes listed in Table 2 it is of particular note that there are a number of SNPs in genes implicated in the control of lung development which also show evidence of association with COPD risk, namely *HHIP* (SHH pathway), *FGF7* (Fibroblast Growth Factor pathway) and *SOX*9 (Wnt/β-catenin pathway).

Table 1 FEV_1 associated SNPs identified using GWAS meta-analyses

Gene	Locus	SNP	Measure	Reference
ST3GAL3	1p34.1	rs121374475	FEV_1 decline	Tang et al. 2014 [28]
NFIA	1p31.3	rs766488	FEV_1 decline	Tang et al. 2014 [28]
ESRRG/GPATCH2	1q41	rs17698444	FEV_1 decline	Tang et al. 2014 [28]
BAZ2B	2q24.2	rs12692550	FEV_1 decline and [b]Rate of FEV_1 change	Tang et al. 2014 [28]
FOSL2/PLB1	2p23.2	rs10209501	[b]Rate of FEV_1 change	Tang et al. 2014 [28]
TNS1	2q35	rs2571445	FEV_1	Repapi et al. 2010 [19]
HDAC4	2q37.3	rs12477314	FEV_1	Soler-Artigas et al. 2011 [36]
MECOM	3q26.2	rs1344555	FEV_1	Soler-Artigas et al. 2011 [36]
FLJ25363/MIR4445	3q13.13	rs1729588	[b]Rate of FEV_1 change	Tang et al. 2014 [28]
GSTCD	4q24	rs10516526	FEV_1	Repapi et al. 2010 [19]
FLJ20184	4q24	46 SNPs across locus	FEV_1	Hancock et al. 2010 [20]
INTS12	4q24	46 SNPs across locus	FEV_1	Hancock et al. 2010 [20]
GSTCD	4q24	46 SNPs across locus	FEV_1	Hancock et al. 2010 [20]
NPNT	4q24	46 SNPs across locus	FEV_1	Hancock et al. 2010 [20]
HHIP	4q31	rs12604628	FEV_1	Repapi et al. 2010 [19]
PDE4D[a]	5q12	rs298028	FEV_1	Obeidat et al. 2011 [22]
HTR4	5q33	rs3995090	FEV_1	Repapi et al. 2010 [19]
ZKSCAN3	6p22.1	rs6903823	FEV_1	Soler-Artigas et al. 2011 [23]
MTHFD1L[a]	6q25.1	rs803450	FEV_1	Obeidat et al. 2011 [22]
NAT2[a]	8p22	rs6988857	FEV_1	Obeidat et al. 2011 [22]
CDC123	10p13	rs7068966	FEV_1	Soler-Artigas et al. 2011 [23]
C10orf112/MALRD1	10p12.31	rs10764053	[b]Rate of FEV_1 change	Tang et al. 2014 [28]
C10orf11	10q22.3	rs11001819	FEV_1	Soler-Artigas et al. 2011 [23]
ME3	11q14.2	rs507211	[b]Rate of FEV_1 change	Tang et al. 2014 [28]
CNTN5[a]	11q22 - q22.2	rs17133553	FEV_1	Obeidat et al. 2011 [22]
TRPV4[a]	12q24.1	rs3742030	FEV_1	Obeidat et al. 2011 [22]
TMCO3	13q34	rs2260722	FEV_1 decline	Tang et al. 2014 [28]
SERPINA1[a]	14q32.13	rs3748312	FEV_1	Obeidat et al. 2011 [22]
IL16/STARD5/TMC3[a]	15q25.1	rs4077833	FEV_1 decline	Tang et al. 2014 [28]
SV2B	15q26.1	rs8027498	FEV_1 decline	Tang et al. 2014 [28]
MYH11	16p13.11	rs8051319	FEV_1 decline	Tang et al. 2014 [28]
CACNG4	17q24.2	rs740557	FEV_1 decline	Tang et al. 2014 [28]
KCNJ2/SOX9	17q24.3	rs11654749	FEV_1	Hancock et al. 2012 [24]
BCL2[a]	18q21.3	rs2850760	FEV_1	Obeidat et al. 2011 [22]
MACROD2[a]	20p12.1	rs204652	FEV_1	Obeidat et al. 2011 [22]

Top hits for FEV_1 association seen in 6 studies of genome-wide association study meta-analyses [19, 20, 22–24, 28]
[a]Top hits that were outside the level of significance, [b]In meta-analyses of 5 cohorts with over 3 measurements per participant

Genetics of lung development

Gene expression across lung development is a complex and intricately timed process. Several signalling pathways in particular are considered key for correct lung development (Table 3). Lung development is also subdivided into five distinct developmental stages (Fig. 2), each governed by specific signalling cascades (Table 4).

The mammalian respiratory system originates from the anterior foregut endoderm in the foetus for the purpose of developing an ideal structure to facilitate gas exchange. During embryogenesis and the following pseudoglandular stage the two lung buds begin a complex process of branching morphogenesis; a highly regulated process generating a tree-like structure of epithelial tubes branching

Table 2 COPD associated SNPs identified using GWAS or candidate gene methodology

Gene	Locus	SNP	Association/Comparison	Reference
TGFB2	1q41	rs4846480	Severe COPD vs healthy smoker	Cho et al. 2014 [34]
TNS1	2q35	rs2571445	COPD susceptibility	Soler-Artigas et al. 2011 [36]
PID1	2q36.3	rs10498230	FEV_1/FVC associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
PID1	2q36.3	rs1435867	FEV_1/FVC associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
PID1	2q36.3	rs16825116	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
FAM13A	4q22.1	rs2869967	Chronic Bronchitis COPD vs smoker control	Lee et al. 2014 [29]
FAM13A	4q22.1	rs2045517	Chronic Bronchitis COPD vs smoker control	Lee et al. 2014 [29]
FAM13A	4q22.1	rs7671167	Chronic Bronchitis COPD vs smoker control	Lee et al. 2014 [29]
FAM13A	4q22.1	rs7671167	Airway obstruction in COPD	Cho et al. 2012 [32]
FAM13A	4q22.1	rs7671167	Emphysema in COPD cohort	Pillai et al. 2010 [30]
FAM13A	4q22.1	rs7671167	COPD susceptibility	Cho et al. 2010 [31]
FAM13A	4q22.1	rs1903003	COPD susceptibility	Cho et al. 2010 [31]
FAM13A	4q22.1	rs2904259	Chronic Bronchitis COPD vs smoker control	Lee et al. 2014 [29]
FAM13A	4q22.1	rs2609264	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
FAM13A	4q22.1	rs2609261	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
FAM13A	4q22.1	rs2609260	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
FAM13A	4q22.1	rs4416442	Moderate to severe and severe COPD vs healthy smoker	Cho et al. 2014 [34]
FAM13A	4q22.1	rs1964516	Airway obstruction in COPD	Cho et al. 2012 [32]
FLJ20184	4q24	rs17035960	FEV_1 associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
FLJ20184	4q24	rs17036052	FEV_1 associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
INTS12	4q24	rs11727189	FEV_1 associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
INTS12	4q24	rs17036090	FEV_1 associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
GSTCD	4q24	rs10516526	COPD susceptibility	Soler-Artigas et al. 2011 [36]
GSTCD	4q24	rs10516526	FEV_1 associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
GSTCD	4q24	rs11097901	FEV_1 associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
GSTCD	4q24	rs11728716	FEV_1 associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
NPNT	4q24	rs17036341	FEV_1 associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
NPNT	4q24	rs17331332	FEV_1 associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
HHIP	4q31.21	rs13141641	Moderate to severe and severe COPD vs healthy smoker	Cho et al. 2014 [34]
HHIP	4q31.21	rs12504628	COPD susceptibility	Soler-Artigas et al. 2011 [36]
HHIP	4q31.21	rs13118928	FEV_1/FVC, Emphysema and Exacerbations in COPD cohort	Pillai et al. 2010 [30]
HHIP	4q31.21	rs13118928	COPD susceptibility	Cho et al. 2010 [31]
HHIP	4q31.21	rs13118928	COPD susceptibility	van Durme et al. 2010 [103]
HHIP	4q31.21	rs1828591	COPD susceptibility	van Durme et al. 2010 [103]
HTR4	5q32	rs7733088	Airway obstruction	Wilk et al. 2012 [35]
HTR4	5q32	rs3995090	COPD susceptibility	Soler-Artigas et al. 2011 [36]
ADAM19	5q33	rs2277027	FEV_1/FVC associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
ADAM19	5q33	rs1422795	FEV_1/FVC associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
PPT2	6p21	rs10947233	FEV_1/FVC associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
AGER	6p21	rs2070600	FEV_1/FVC associated measure - COPD susceptibility	Castaldi et al. 2011 [96]
ACN9	7q21.3	rs10231916	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
ACN9	7q21.3	rs10229181	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
RHOBTB1 - TMEM26	10q21.2	rs10761570	Decline in FEV_1 in mild/moderate COPD	Hansel et al. 2013 [104]
RHOBTB1 - TMEM26	10q21.2	rs7911302	Decline in FEV_1 in mild/moderate COPD	Hansel et al. 2013 [104]

Table 2 COPD associated SNPs identified using GWAS or candidate gene methodology *(Continued)*

EFCAB4A	11p15	rs34391416	Chronic Bronchitis COPD vs smoker control	Lee et al. 2014 [29]
CHID1	11p15	rs147862429	Chronic Bronchitis COPD vs smoker control	Lee et al. 2014 [29]
ANO3	11p14.2	rs7119465	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
MMP12	11q22.2	rs626750	Severe COPD vs healthy smoker	Cho et al. 2014 [34]
BICD1	12p11.21	rs10844154	Emphysematous COPD	Kong et al. 2011 [105]
BICD1	12p11.21	rs161976	Emphysematous COPD	Kong et al. 2011 [105]
LOC100128066 - TTC6	14q21.1	rs177852	Decline in FEV_1 in mild/moderate COPD	Hansel et al. 2013 [104]
RIN3	14q32.12	rs754388	Moderate to severe and severe COPD vs healthy smoker	Cho et al. 2014 [34]
IREB2	15q25.1	rs1062980	COPD susceptibility	Brehm et al. 2011 [37]
IREB2	15q25.1	rs13180	COPD susceptibility	Brehm et al. 2011 [37]
IREB2	15q25.1	rs8034191	COPD susceptibility	Brehm et al. 2011 [37]
IREB2	15q25.1	rs265606	COPD susceptibility	Brehm et al. 2011 [37]
PSMA4	15q25.1	rs2036534	COPD susceptibility	Brehm et al. 2011 [37]
AGPHD1 - CHRNA3/5	15q25.1	11 SNPs	Airway obstruction in ever smoker	Wilk et al. 2012 [35]
CHRNA5	15q25.1	rs17486278	Airway obstruction in all ever/never smoker	Wilk et al. 2012 [35]
CHRNA3/5	15q25.1	rs8034191	FEV_1, FEV_1/FVC and Emphysema in COPD cohort	Pillai et al. 2010 [30]
CHRNA3	15q25.1	rs12914385	COPD susceptibility	Brehm et al. 2011 [37]
CHRNA3	15q25.1	rs1051730	COPD susceptibility	Brehm et al. 2011 [37]
CHRNA3	15q25.1	rs12914385	Moderate to severe and severe COPD	Cho et al. 2014 [34]
CHRNA3/CHRNA5/IREB2	15q25.1	rs1062980	COPD susceptibility	Cho et al. 2010 [31]
CHRNA3/CHRNA5/IREB2	15q25.1	rs13180	COPD susceptibility	Cho et al. 2010 [31]
CHRNA3/5	15q25.1	rs8034191	COPD susceptibility	Pillai et al. 2009 [38]
CHRNA3/5	15q25.1	rs1051730	COPD susceptibility	Pillai et al. 2009 [38]
FGF7	15q21.2	rs12591300	COPD susceptibility	Brehm et al. 2011 [37]
FGF7	15q21.2	rs4480740	COPD susceptibility	Brehm et al. 2011 [37]
DTWD1	15q21.2	rs17404727	COPD susceptibility	Brehm et al. 2011 [37]
MCTP2	15q26.2	rs8031759	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
AKAP1	17q22	rs886282	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
SOX9	17q24.3	rs17178251	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
SOX9	17q24.3	rs17765644	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
SOX9	17q24.3	rs11870732	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]
RAB4B - EGLN2	19q13	rs7937	Airway obstruction in COPD	Cho et al. 2012 [32]
RAB4B - EGLN2	19q13	rs2604894	Airway obstruction in COPD	Cho et al. 2012 [32]
PDE9A	21q22.3	rs2269145	Lung function gene associated with COPD susceptibilty	Kim et al. 2014 [33]

COPD gene associations in the literature [29–38, 96, 103–105]

by dichotomy [39]. Branching is driven by a number of signalling pathways communicating between the mesenchyme and the epithelium, directing the growth and patterning of lung buds (Table 3). Branching morphogenesis is a critical time during lung development determining lung resistance and compliance in adult life. As discussed above, these determinants of airway function can be quantified by FEV_1 and FEV_1/FVC measures, and therefore polymorphic variation in genes active during the period of airway branching could feasibly be linked to adult lung function [40].

Of the highly complex signalling systems; Sonic Hedgehog (SHH) and Fibroblast growth factor (FGF) are considered two of the primary signalling pathways critical for lung development [39]. The critical role of separation of the trachea from oesophagus is influenced by SHH signalling and FGF patterning, with both pathways initially involved in determining distal airway development [41, 42]. Furthermore, the transcription factor Nkx2.1 marks the future oesophagus and Wnt signalling works alongside to specify lung fate [43]. In relation to lung diseases, despite regeneration and repair of injured

Table 3 Key signalling pathways involved in mammalian lung development

Signalling pathway	Role in lung development
Fibroblast Growth Factor (FGF)	Role in the rate of airway bud extension
	Involved in the formation of new alveoli
	Limits proliferation or migration of branching epithelium
	FGF among the signals that confer anterior-posterior identity
Sonic Hedgehog (SHH)	Important in regulating lung cell proliferation and asymmetry
	Negatively regulates FGF10
	Limits lung bud growth
NK2 homeobox (NKX2.1)	Important in formation of tracheo-oesophageal septum
	Essential for initiating branching
Notch	Key role in cell-cell communication during lung development
	Promotes proximal lung cell fates
Planar Cell Polarity (PCP)	Drives polarisation of cells
	Required for branching
	Involved in determining lung architecture
Retinoic Acid	Promotes growth of the primary lung buds
	Down-regulates TGF β signalling
Transforming growth factor β/Bone morphogenic protein (BMP)-SMAD (TGFβ/BMP-SMAD)	Can inhibit/stimulate lung morphogenesis
	Contributes to distal lung development
	BMP among the signal that confer anterior-posterior identity
Wnt/β-catenin	Negatively regulates branching
	Involved in developing peripheral airways

Lung development and signalling pathway information was collected from many sources [39, 42, 43, 106–120]

lung tissue not currently being fully understood, it can be hypothesised that events would follow the same or similar pathways as those used during lung development outlined here. Therefore, it is important to understand any potential associations between genes involved in both COPD and lung development. For instance, of the genes associated with COPD in Table 2, *SOX9, HHIP, MMP12, HTR4* and *FGF7* also have distinct roles during lung development. *SOX9, HHIP, FGF7* are involved in airway branching morphogenesis typically with expression levels peaking during the embryonic and pseudoglandular stages [44–53]. *SOX9* expression can be modulated by a number of key pathways including: HH, Wnt/β-catenin, Notch, TGF-β, NFKB, BMP and FGF [54]. Additional genes of interest due to varying expression patterns across and associations with lung development include: *EFCAB4A, CHID1, ANO3, AKAP1, TGFβ2, GSTCD* and *NPNT* [20, 21, 55–60]. These genes have been demonstrated to be significantly associated with COPD (Table 2) and show preliminary evidence for involvement with lung development, with a common feature of varied expression during branching morphogenesis stages. SNPs in the genes *AGER, HHIP* and *TNS1* are associated with reduced airway calibre and may be involved in lung development and growth [47]. In summary therefore, it appears that many of the genes which potentially underlie the associations seen in GWA studies of lung function and/or COPD are involved in control of lung development and potentially remodelling. Some additional support for a role in lung development comes from the observation that associations with lung function are present across the age spectrum, although the number of studies in younger age groups and children to date has been small. Furthermore, the mechanism of action of potential susceptibility genes can vary, where genetic susceptibility could lead to dysregulated lung development (as discussed) during childhood or adolescence or may lead to enhanced decline of FEV_1 in adulthood, which has long been considered the most common indicator of COPD [61–63]. A recent study has indicated that approximately half of the individuals who meet the criteria for COPD in later life (COPD at grade 2 or higher according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) grading system) [4] do attain normal maximal FEV_1 in young adulthood and have accelerated rates of decline [64]. However, the authors also suggest that a substantial proportion of patients with COPD may not have had a rapid decline in FEV_1, as the second half of participants followed a more typical decline in FEV_1 starting from a low initial value of

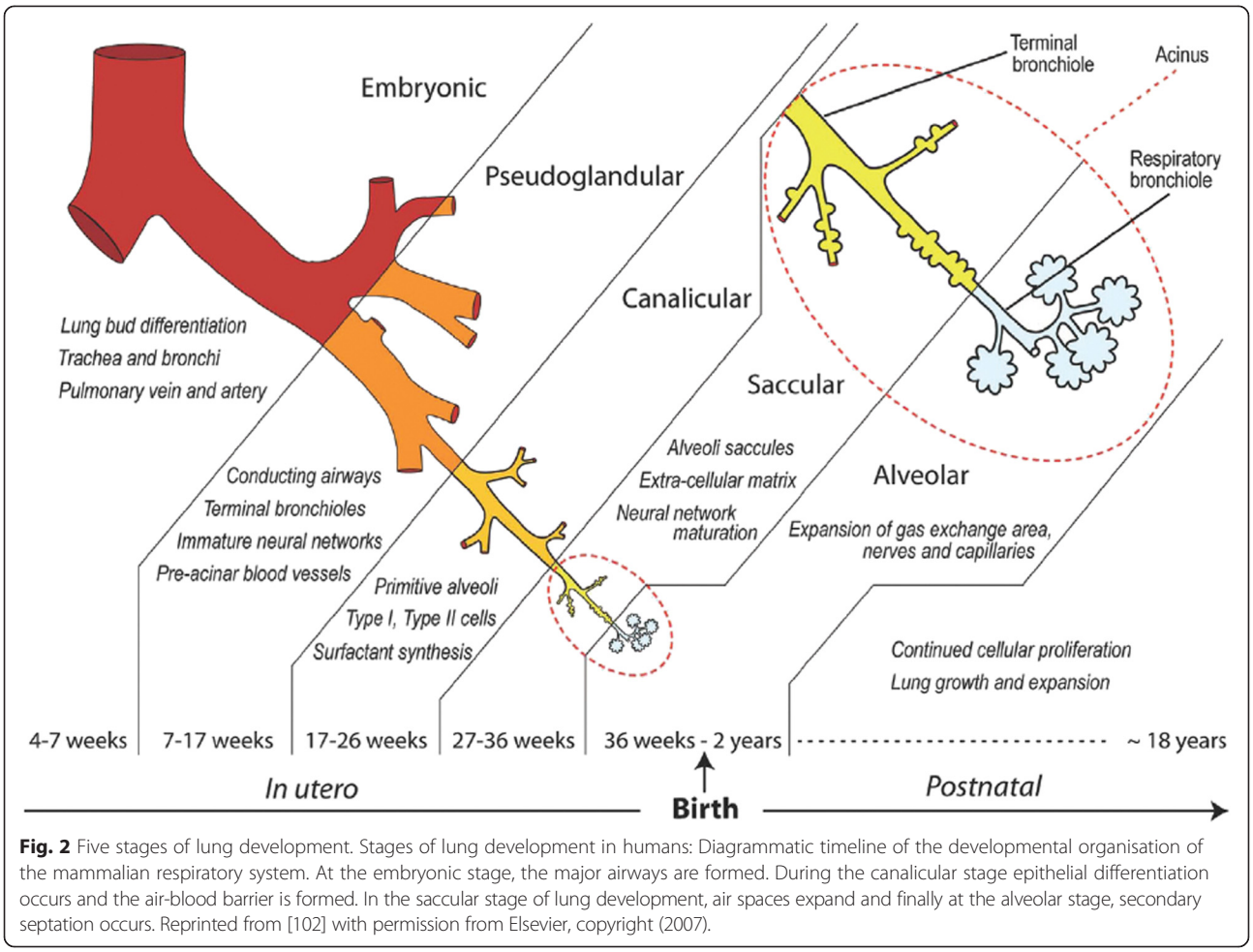

Fig. 2 Five stages of lung development. Stages of lung development in humans: Diagrammatic timeline of the developmental organisation of the mammalian respiratory system. At the embryonic stage, the major airways are formed. During the canalicular stage epithelial differentiation occurs and the air-blood barrier is formed. In the saccular stage of lung development, air spaces expand and finally at the alveolar stage, secondary septation occurs. Reprinted from [102] with permission from Elsevier, copyright (2007).

FEV_1. Hence this may indicate populations of COPD patients with different rates of decline in FEV_1 potentially a consequence of dysregulated lung development or an earlier rapid decline in FEV_1 [64]. Additionally, it can be reasoned that the most important determinant of maximally attained lung function later in life is lung function measurement at a younger age, as shown by several studies involving children and young adults [65–68] which may indicate initial dysregulated lung development.

Also, in 2004, de Marco et al. have proposed that the origin of COPD could be from an earlier age group than is usually believed, as a considerable percentage of subjects aged 20–44 years reported already suffering from COPD and GOLD stage 0 chronic symptoms [69] and later the group identified a subgroup of young adult subjects with a high risk of developing COPD, independently of smoking habits [70].

Epigenetics considerations in lung function and COPD

Epigenetics is commonly defined as heritable changes to gene expression, independent of changes to DNA sequence. Whereas genetic changes in DNA sequence involve variation of nucleotides, epigenetic changes alter methylation patterns at CpG sites or modifications to chromatin, influencing the level of DNA folding and therefore the levels of transcription at a particular gene. This area of research investigates the link between lifetime exposures of parents with the influence these may have on epigenetic patterns in children. Despite epigenetics consisting of dynamic and modifiable processes which can change over time, it is of key interest as these changes can persist across generations [71].

Typically, COPD is classed as a disease of later life, although as discussed above predisposition to COPD may also have an early origin during lung development. In particular, smoking during pregnancy has been investigated to understand the effects of smoking exposure on lung development, as it is suggested that susceptibility to environmental factors is highest during this period and changes may contribute to adult airflow limitation [72]. Furthermore, maternal smoking has been demonstrated to be associated with lower adult lung volume independent to post-natal exposure and of personal

Table 4 Stages and events during lung development in humans and mice

Developmental stage	Human gestation age	Mouse gestation age	Lung development events
Embryonic	4-7 weeks	9-14 days	Septation of trachea from oesophagus
			Lung bud forms two main bronchi and individual lobes
			Supporting structures are formed including bronchial cartilage and smooth muscle
Pseudoglandular	5-17 weeks	14-16.5 days	Branching morphogenesis begins
			Proximal airway epithelial and mesenchymal differentiation occurs
Canalicular	16-26 weeks	16.5-17.5 days	Further branching
			Vascularisation and angiogenesis occurs along the airway
			Rapid increase in capillary numbers
			Respiratory bronchioles and alveolar ducts form
			Type II alveolar epithelial cells differentiate
Saccular	24-36 weeks	17.5 days to postnatal day 5	Type I alveolar epithelial cells differentiate
			Type II alveolar epithelial cells maturation
			Air sacs are developed
			Lymphatic network develops
			Surfactant begins to be produced
			Initial stage of primary septation for formation of alveoli occurs
Alveolar	36 weeks to childhood	Postnatal day 5-30	Secondary septation produces alveoli
			Increasing levels of surfactant produced
			Majority of gas exchange surface is formed

Information on the stages of human and mouse lung development was collected from many sources [42, 107, 121, 122]

smoking [72–76]. Of the wide range of components in tobacco smoke nicotine is thought to be the key component which alters lung development, principally because it is easily transferred to the foetus *in utero* in circulating blood [77–81]. Importantly, approximately 12-22 % of women smoke during pregnancy [82–87]. Data from animal studies and observations in humans show that smoking during pregnancy is associated with lower lung function in offspring and increases in airway smooth muscle, decreasing alveolar surface area and collagen deposition [78, 79, 88, 89]. Effects influencing lung function such as these can be attributable to epigenetic changes which may lead to a predisposition to developing COPD. For instance, exposure to nicotine *in utero* has been demonstrated to increase DNA methylation and acetylation in the foetus, which would be predicted to produce down-regulation and up-regulation of transcriptional activity, respectively, in the relevant target genes [77].

However, few studies have been performed identifying alterations at specific epigenetic markers in response to maternal smoking and COPD. Nevertheless, an interesting direction may be in the form of altered methylation patterns in repetitive elements across the genome. In 2009, Breton et al. demonstrated that pre-natal smoking has been associated with methylation patterns in repetitive elements, such as AluYb8, also in conjunction with null genotypes in genes involved in tobacco smoke metabolism (*GSTM1* and *GSTP1*) [90]. This study suggests differential methylation changes may potentially be dependent upon the genotype of a foetus, hence determining the level of susceptibility to smoke induced epigenetic alterations [90]. The group also showed that smoking during pregnancy was associated with global hypomethylation, suggested to lead to chromosomal instability [90].

With the growing interest in nicotine replacement therapy (NRT) as a seemingly healthier alternative to smoking, the evidence outlined here is a reminder that use of NRT may not be a safe alternative to smoking during pregnancy [91, 92], as NRT would still be predicted to exert epigenetic effects which could alter lung development. Furthermore, maternal smoking has been found to synergise with personal smoking to increase airflow limitation and risk for development of COPD [75].

Characterisation of *INTS12*, *GSTCD* and *HTR4*: examples of genes with potential roles in lung development

We have recently provided evidence indicating the possible role of genetic variation near or at the integrator complex subunit 12 (*INTS12,* 4q24), as influencing lung function measures [21]. We reported that there is a

significant positive correlation between *INTS12* expression in lung tissue and percent predicted FEV_1. The same was true for the nearby Glutathione S-transferase, C-terminal domain containing gene, *GSTCD,* and we hypothesised that these genes share the same promoter region due to the fact that they are co-ordinately transcribed. The two genes are also co-expressed in cells of the lung and whole lung tissue. Interrogation of the publically available ENCODE dataset revealed that the presumed shared promoter contains CpG islands as well as transcription factor binding sites. Most importantly, SNPs that are genome-wide significant for lung function are in cis-eQTL with *INTS12* expression in various tissue types and this was not observed for *GSTCD* nor for any gene in strong linkage disequilibrium (LD) with *INTS12*. By immunohistochemistry of fixed human sections, we have previously shown that GSTCD protein expression was ubiquitous, whereas INTS12 expression was predominantly in epithelial cells and pneumocytes. During human fetal lung development, GSTCD protein expression was observed to be highest at the earlier pseudoglandular stage (10–12 weeks) compared with the later canalicular stage (17–19 weeks), whereas INTS12 expression levels did not alter throughout these stages. Although this work demonstrates potential roles of *INTS12* and *GSTCD* as drivers of the association signal for lung function, much more work is required to ultimately bridge the gap between the 4q24 GWA study findings and how these influence lung function. A separate gene our research group has actively studied is the lung function and COPD associated serotonin receptor, *HTR4*. We identified that the protein level of HTR4 increased throughout lung development; however HTR4 was expressed only at very low mRNA and protein levels in adult lung [50], again suggesting a potential role in lung development.

Models to study candidate lung function/COPD genes: new approaches

As we have noted, although GWA studies have been successful at detecting genomic loci harbouring variants predicting variation in lung function measures and risk of COPD, these genetic associations are usually limited to identifying fairly broad genomic regions and are incapable of distinguishing causal variants from non-causal variants [93]. Therefore despite the unprecedented success of GWA studies, the therapeutic and functional translation of these studies is still in its infancy. There are a number of experimental approaches and models that may be used to functionally translate genetic findings. These methods can help in dissecting the genetic association signals for the currently considered respiratory phenotypes and identify underlying alleles and biological pathways that are important in lung function and COPD. Computational methods can be used to combine experimentally generated regulatory information of the human genome, such as ChIP-seq (chromatin immuno-precipitation sequencing) generated binding sites or gene expression Quantitative Trait Loci (eQTL), with respiratory loci [93, 94]. The classical scheme of following up GWA study associations concentrates on manipulation of single genes (for example generating transgenic mice which have the gene deleted or overexpressed) but this method is inevitably slow. However, given genetic association data suggests the presence of a multitude of gene variants on different chromosomes predicting the disease risk or lung function measure outcome [7, 19, 36, 95, 96]. Recently, the development of the CRISPR-Cas9 activation system, which allows simultaneous enhancement of endogenous expression of multiple genes, may speed up functional follow up of key genetic variants [97]. Additionally, enhancing endogenous gene expression from a natural promoter is more likely to recapitulate the splicing complexity than the traditional transient or stable recombinant DNA transfection approach [97]. RNA interference (RNAi) gene silencing has successfully been used to knock down genes of interest and following downstream analyses, novel gene functions have been identified. However, with RNAi-based approaches, the data requires in depth complex analysis. Ideally, more than one siRNA or shRNA could be utilised due to the degree of false positive observations, which may obscure true results with off-target silencing effects [98]. This limitation can also be addressed with the advent of CRISPR and TALEN gene editing technology which allows generation of specific gene knock-out cells with the potential for several individual gene knock-outs in combination [99]. Of note, decreases in the cost of next generation high-throughput sequencing has addressed a number of limitations faced by microarray-based approaches and allows effective discovery of biological pathways underpinning respiratory phenotypes, for example by RNA-sequencing and CHIP sequencing approaches [100]. This information could be used to make informed decisions about relevant cellular assays *post* genetic manipulation. Investigating respiratory phenotypes in lung tissue from specific gene knockout mice is also a valuable in vivo approach that can effectively complement *in vitro* work [101].

Conclusions

In conclusion, recent advances in large GWA studies and meta-analysis of results obtained across different studies has led to the identification of a large number of loci which predict lung function variability. An increasing number of these loci have also been demonstrated to show association with COPD risk *per se*. However, despite these advances, only a small proportion of the variability in lung function can be explained by the genetic

variants described to date. This suggests many other variants are yet to be uncovered which may also contribute to the genetic basis of airflow obstruction. It is notable that many of the genetic regions which have been identified to date harbour genes which play an important role in lung development. Whether or not this means these genes are less likely to be useful targets for therapeutic manipulation remains to be defined. However, there is no doubt that understanding the role of these genes in the regulation of lung function will be key to improving our knowledge of the pathophysiology of COPD and other diseases characterised by airflow obstruction.

The observation that genes associated with lung function and COPD and also showing evidence of differential expression during lung development makes them good candidates playing critical roles in embryological lung development. However, more studies are warranted to demonstrate that through carefully controlled experiments SNP mutagenesis in those genes or whole gene knockout models display effects on lung morphogenesis or activity. If shown to be the case it would give more credence to the 'Dutch hypothesis' stating that COPD and asthma are essentially different manifestation of the same disease process. This is because originally this hypothesis was based on the observation that there is a fluent development from bronchitis in youth to a more asthmatic picture in adults which then further develops into bronchitis among more elderly patients. Therefore existence of genetic variants predisposing to pathobiology of lung development may be expected under this scenario.

Abbreviations

ChIP-Seq: chromatin immunoprecipitation sequencing; COPD: chronic obstructive pulmonary disease; CRISPR: clustered regularly interspaced short palindromic repeats; ENCODE: Encyclopedia of DNA Elements; eQTL: expression Quantitative trait loci; FEV_1: forced expiratory volume in 1 second; FGF: Fibroblast growth factor; FVC: forced vital capacity; GWA: genome-wide association; GWAS: genome-wide association study; NRT: nicotine replacement therapy; SHH: Sonic Hedgehog; SNP: single nucleotide polymorphism; TALEN: transcription activator-like effector nuclease.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SM and IPH planned the content of the review, KP, SM and AKK searched and reviewed the literature and drafted the review. All authors contributed to the final version. All authors read and approved the final manuscript.

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