



ORIGINAL ARTICLE

Fibroblast growth factor 10 haploinsufficiency causes chronic obstructive pulmonary disease

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ABSTRACT

Background Genetic factors influencing lung function may predispose to chronic obstructive pulmonary disease (COPD). The fibroblast growth factor 10 (FGF10) signalling pathway is critical for lung development and lung epithelial renewal. The hypothesis behind this study was that constitutive FGF10 insufficiency may lead to pulmonary disorder. Therefore investigation of the pulmonary functions of patients heterozygous for loss of function mutations in the FGF10 gene was performed.

Methods The spirometric measures of lung function from patients and non-carrier siblings were compared and both groups were related to matched reference data for normal human lung function.

Results The patients show a significant decrease in lung function parameters when compared to control values. The average FEV1/VC quota (FEV1%) for the patients is 0.65 (80% of predicted) and reversibility test using Terbutalin resulted in a 3.7% increase in FEV1. Patients with FGF10 haploinsufficiency have lung function parameters indicating COPD. A modest response to Terbutalin confirms an irreversible obstructive lung disease.

Conclusion These findings support the idea that genetic variants affecting the FGF10 signalling pathway are important determinants of lung function that may ultimately contribute to COPD. Specifically, the results show that FGF10 haploinsufficiency affects lung function measures providing a model for a dosage sensitive effect of FGF10 in the development of COPD.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a complex disease and one of the leading causes of death in developed countries. The disease results from major remodelling of the airspace and changes in the lung epithelium.¹ The simplest classification of COPD is related to the degree of airflow obstruction, regardless of the underlying mechanism. In contrast to asthma, which is defined by a reversible airflow obstruction, COPD is characterised by irreversible and usually progressive obstruction with an abnormal inflammatory response to noxious gases or particles.^{2–3} The most important risk factor for the development of COPD is cigarette smoking, but only a minority of smokers will develop the disease, suggesting contributions of genetic factors.⁴ This is supported by the demonstration of familial aggregation of lung function measures as well as the clustering of COPD within families.⁵ Genetic studies in humans and in animal models suggest that genes associated

with lung developmental processes are also implicated in COPD.^{1–4} Lung development requires branching morphogenesis, a process that is dependent on cell–cell communication between the mesenchyme and the lung epithelium. Both lung development and lung homeostasis depend on precise molecular signalling involving different fibroblast growth factors (FGF), sonic hedgehog (SHH), bone morphogenic protein (BMP), transforming growth factor β (TGF β), retinoic acid, the wntless (WNT) related family of proteins, and various transcription factors.^{7–8} Similar mechanisms are required to maintain and regenerate the lung epithelium from progenitor cells located both in the epithelium and in the mesenchyme along the airways.⁹ Thus, factors and molecular pathways involved in the differentiation and renewal of the lung epithelium provide candidate mechanisms in pulmonary disease.^{10–11}

FGF10 is expressed in the mesenchyme and its signalling is mediated by the receptor FGFR2b required for the development of many branched organs including lungs, thyroid, pituitary, lacrimal, and salivary glands.^{12–14} During early lung development, FGF10 signalling is tightly regulated by the retinoic acid dependent network through either WNT (via DKK1) or TGF β -1 signalling.⁸ In the developing lung FGF10 is regulated by SHH signalling and other members of the FGF family.^{15–16} Furthermore, decreased FGF10-FGFR2 signalling alters the expression of SHH and BMP4, as well as FGF10 itself, establishing a feedback loop.¹⁷ The importance of the FGF10 pathway during lung initiation and development has been established in model systems after targeted disruption of the corresponding genes or by ectopic over-expression of the proteins.^{18–20} The most profound disruption of lung morphogenesis is observed in *Fgf10* and *Fgfr2* null mice embryos showing failure to form lung buds.^{21–24} In the adult lung, FGF10 has been identified as a survival factor, and exogenous *Fgf10* expression in mice decreases induced alveolar epithelial cell DNA damage, apoptosis, and the extent of induced lung fibrosis.^{25–26} Furthermore, FGF10 is coordinately expressed with BMP4 during hyperoxia injury repair.²⁷ In vitro studies have recently shown that soluble FGF10 and hepatocyte growth factor (HGF) can replace the requirement for mesenchymal support in lung epithelial formation, allowing for clonal expansion and self-renewal.⁹ These data support a model in which the adult lung contains FGF10 dependent cells, giving rise to airway and alveolar epithelial cell lineages.

In humans, heterozygous mutations in the genes encoding FGF10 or FGFR2b result in autosomal dominant aplasia of lacrimal and salivary glands (ALSG) and lacrimo-auriculo-dento-digital syndrome (LADD), respectively. No lung phenotype has been described in these patients, with the exception of a male LADD patient presenting with complex pulmonary malformations.^{28–32} FGF10 and FGFR2b are essential for lung initiation, lung development, and lung epithelial proliferation in experimental systems, and we hypothesised that haploinsufficiency for FGF10 affects lung function that may ultimately result in the development of COPD.³³ To examine this hypothesis, we measured the pulmonary functions in patients with loss of function mutations in the *FGF10* gene as well as their non-carrier siblings. Spirometric values from both groups were compared with reference data for pulmonary functions.³⁴ Lung function parameters were also measured in a mice model heterozygous for *Fgf10* in order to validate a pulmonary phenotype. Our results indicate that *FGF10* haploinsufficiency affects lung function that may ultimately lead to the development of COPD.

METHODS

Patients

Twelve patients with ALSG and heterozygous loss of function mutations in *FGF10* were included in this study. The patients belong to two previously described families segregating a deletion (n=10) or a nonsense mutation (n=2) in the *FGF10* gene, respectively.²⁹ The age range of patients was from 13–69 years, with a mean age of 39 years. Six patients were male and six female. Six of the 12 patients were diagnosed with ‘asthma’ before this study and four patients had milder allergies. Two were smokers (<one package a day), three patients stopped smoking 3–11 years ago, and seven patients never smoked (supplemental table S1). None of the patients had any known lung malformation or abnormal tendency for airway/lung infections. Three non-carrier siblings (age 30, 33 and 34 years, one male and two females, respectively) from one family were included as ‘internal’ controls. Informed consent was obtained from all adult participants or parents of children who participated in this study under a protocol approved by the Regional Ethical Committee.

Mice

Mice heterozygous for the *Fgf10* gene²² were housed and bred in the animal core facility of the Biomedical Centre, Uppsala University under pathogen-free conditions. In total, 12 haploinsufficient (*Fgf10* +/-) mice and 12 wt (*Fgf10* +/+) mice (C57BL/6) were used in this study at 13–17 weeks of age. Genotyping was performed using a primer against the neo-cassette together with a specific mouse *Fgf10* primer (available upon request). The study followed a protocol approved by the Regional Ethical Committee for animal experiments.

Pulmonary function

Lung function measurements of study participants were performed by trained technicians and according to the American Thoracic Society (ATS) or European Respiratory Society (ERS) criteria.³⁵ Lung function in mice were investigated using the eSpira Forced Manoeuvres System (FMS) (EMMS, Hants, UK). *Human*: We measured forced expiratory volume during 1 s (FEV₁) and vital capacity (VC) using a computerised pneumotachograph spirometer. Inspiratory vital capacity (IVC) was measured and total lung capacity (TLC) calculated with a volume plethysmograph (Jaeger Masterscreen body; Hoehberg, Germany). FEV₁ was determined after treatment with

terbutaline. *Mouse*: Mice were anaesthetised (0.2 ml/20 g mouse, intraperitoneally, with ketamine, xylazine, acepromazine, and saline, 2:1:0.3:6 ml). The eSpira FMS was used to assess the lung function parameters forced expiratory volume in 75 ms (FEV₇₅), forced vital capacity (FVC), inspiratory capacity, and TLC.

Reference data

Normal reference values for FEV₁ were obtained from a published dataset of spirometry from childhood to old age.³⁴ The references for normal human lung function are based on measures from an ethnically matched control population (sex, age, height). Individual reference values (predicted values) for TLC were calculated according to the following formulae: TLC (l) males=(7.956* (height (m))–6.948, RSD=0.77, TLC (l) females=(7.107* (height (m))–6.43, RSD=0.53.³⁶ The reference values are comparable to previously published values from the Swedish population.^{37–39} The lower limit of the normal (LLN) range was calculated by subtracting 1.645 * RSD (residual SD) from the predicted value.

Statistics

Spirometry data from *FGF10* heterozygous patients were compared with the predicted reference values using paired Student t test assuming equal variance, as well as to the values obtained from three siblings without *FGF10* mutations using two-tailed Student t test assuming equal variance. Data from the healthy siblings were compared with predicted reference values using two-tailed Student t test assuming equal variance to validate the use of the prediction equations. Airway obstruction was classified according to the ATS/ERS 2005 (obstruction FEV₁/VC <LLN) and the GOLD 2006 (FEV₁/VC <0.70) standards.^{2, 40} The use of a single cut-off for FEV₁/VC of 0.70 (GOLD 2006) to define the limits of normal of lung function, without considering age, may result in a false negative diagnosis of obstruction before age 45–50 years as well as a false positive diagnosis above this age because of the effect of age on lung volumes. A method used to minimise this potential misclassification, as supported by both the ATS and the ERS, is the LLN values for FEV₁/VC (ATS/ERS 2005).⁴¹ This is based on the normal distribution and classifies the bottom 5% of the healthy population as abnormal. We classified the patients according to both standards (supplementary table S2). The influence of smoking on pulmonary functions (FEV₁, IVC, and FEV₁/IVC according to Tiffeneau index⁴²) was assessed with two-way ANOVA and two-tailed Student t test, assuming equal variance, by grouping individuals as non-smokers, current smokers, and former smokers. The group of non-smokers was compared to current smokers and former smokers; non-smokers and former smokers were compared to current smokers, and non-smokers were compared to current smokers.

The data from wt (*Fgf10* +/+) mice were compared to heterozygous (*Fgf10* +/-) littermates using two-tailed Student t test assuming equal variance. Comparison of lung measures between the two groups of mice was performed with and without normalisation to body weight (BW), lung weight (LW) or lung capacity (total lung capacity TLC). Values of p<0.05 were considered significant.

RESULTS

Lung function in human subjects

The *FGF10* heterozygous patients showed a significant decrease in IVC (83% of predicted; p<0.001), FEV₁ (68% of predicted; p<0.001), and FEV₁/IVC quota (FEV₁% 0.65; p<0.001), but not in TLC (93% of predicted; p=0.12) (table 1 and table S2).

Table 1 Spirometric characteristics of *FGF10* heterozygous individuals and three non-carrier siblings with predicted reference values

Parameter	Patients (n=12)	Predicted (LLN)† (n=12)	% of predicted	Paired t test	Controls (n=3)	Predicted (LLN)† (n=3)	% of predicted	Paired t test
IVC	3.94±0.97	4.50 (3.60)±0.84 (0.68)	82%±14%	0.0038*	4.56±0.74	4.69 (3.86)±1.16 (0.96)	99%±9%	0.71
FEV ₁	2.56±0.70	3.63 (2.87)±0.60 (0.50)	68%±11%	7.88×10 ⁻⁸ *	3.76±0.74	3.85 (3.76)±0.87 (0.74)	98%±4%	0.41
FEV ₁ /IVC	0.65±0.06	0.82 (0.71)±0.06 (0.06)	80%±7%	1.86×10 ⁻⁶ *	0.82±0.06	0.83 (0.82)±0.02 (0.06)	99%±9%	0.84
TLC	6.01±1.43	6.46 (5.39)±1.11 (0.92)	93%±14%	0.12	6.36±1.47	6.19 (5.19)±1.39 (1.17)	103%±5%	0.42

*Denote significant difference of $p < 0.05$ using two-tailed paired t test.

†Predicted reference values and lower limit of normal (LLN) according to Stanojevic *et al* (2008) or Roberts *et al* (1991).³⁶

Plus-minus values are means±SD.

BMI, body mass index; IVC, inspiratory vital capacity; FEV₁, forced expiratory volume in 1 s; TLC, total lung capacity.

Significant differences in FEV₁ and FEV₁/IVC were also observed when comparing *FGF10* heterozygous patients and the three non-carrier siblings used as controls (table 2). The values of the non-carrier siblings were similar to their predicted values, validating them as 'internal' controls in the study (table 1). Consistent with pulmonary obstructive disease, the average FEV₁/IVC was reduced (<0.7 and $<LLN$) and the FEV₁ ($<80\%$ of predicted) and a normal, or slightly reduced IVC (IVC $>80\%$ of predicted; table 1). Reversibility test using terbutaline gave a significant increase in FEV₁ (3.7% of predicted value, $p=0.0013$; table 2). This was still subnormal when compared to the predicted reference value (2.70±0.73 vs 3.63±0.60 (LLN 2.87±0.50); tables 1 and 2). Smoking, or having a history of smoking, did not correlate with lower IVC, FEV₁ or FEV₁/IVC in our group of patients (supplementary tables S1 and 2). Individual data revealed two individuals (MS90 and GA80) classified as normal according to the GOLD standard, but they could be defined as false negatives when applying the ATS/ERS 2005 standard (supplementary table S2). On the other hand, the two oldest individuals (IA37 and BA45) were classified as COPD according to the GOLD standard but could be considered as false positive using the ATS/ERS 2005 standard. Individual BA45 was 'borderline' normal with an FEV₁/IVC of 0.65 (LLN 0.64), while individual IA37 could be classified as having a restrictive phenotype with a normal FEV₁/IVC (0.66 $>LLN$ 0.62), due to a very low IVC (2.86 $<LLN$ 3.46), and a low TLC (6.05 $<LLN$ 6.11). Individual LA41 had an obstructive disease (FEV₁/IVC 0.627 $<LLN$ 0.632) with restrictive components (IVC 3.35 $<LLN$ 3.71, and TLC 5.11 $<LLN$ 6.27), indicating a mixed phenotype.

Lung function in mouse model

Fgf10 +/- mice showed a significant decrease in FEV₇₅, FVC, as well as a decrease in the FEV₇₅/FVC quota (FEV₇₅%). The mice also had a decreased lung size (TLC) as well as body and lung

weight compared to wild type (wt) male littermates. However, the lung/body weight ratio was similar when comparing *Fgf10* +/- and *Fgf10* +/- mice. To eliminate a possible effect of body and lung size, mice data were normalised to lung capacity, body weight, lung weight, and by comparing weight matched mice. When comparing weight matched *Fgf10* +/- and *Fgf10* +/- mice we observed a slight decrease in lung weight (supplementary table S3). Lung morphology and histopathology (giemsa staining) appeared normal in *Fgf10* +/- mice (supplementary figure 1).

DISCUSSION

Independent genome-wide association studies (GWAS) have recently identified single nucleotide polymorphisms (SNPs) near the hedgehog interacting protein (*HHIP*) locus on chromosome 4 associated with airflow obstruction.⁴⁵⁻⁴⁶ *HHIP* is a critical negative regulator of the hedgehog pathway, including the ligand sonic hedgehog (*SHH*). Overexpression of *Shh* in mouse lung rudiments reduces transcription of the *Fgf10* gene.¹² Similarly to mice deficient for *Fgf10*, branching morphogenesis is greatly impaired in mice deficient for *Hhip* and it has been hypothesised that the lung phenotype in *Hhip* deficient mice results from hyperactive *Shh* followed by inhibition of *Fgf10* expression.¹⁷ This may explain the morphological similarities in mouse models deficient for either *Hhip* or *Fgf10* and supports altered *FGF10* expression as a candidate mechanism in COPD.²⁰ Interestingly, the *FGF10* downstream target, *STAT V.3*, has recently been associated with COPD, providing yet another link to *FGF10* signalling.⁴⁷

The results of our study show that haploinsufficiency for *FGF10* is associated with reduced pulmonary function consistent with COPD.² *FGF10* insufficient patients show a non-reversible airway obstruction when compared with both predicted reference values and siblings with normal *FGF10*

Table 2 Physical and spirometric characteristics of *FGF10* heterozygous individuals and non-carrier siblings

Parameter	Patients (n=12)	Paired t test†	Controls (n=3)	Paired t test†	Student t test
Age (years)	39±21		32±2		0.623
Length (cm)	174.2±8.6		172.7±11.2		0.802
Weight (kg)	73.8±18.7		72.0±1.7		0.871
BMI	24.1±4.4		24.4±3.6		0.903
IVC	3.94±0.97		4.56±0.74		0.320
FEV ₁	2.56±0.70		3.76±0.74		0.020*
FEV ₁ /IVC	0.65±0.06		0.82±0.06		1.86×10 ⁻⁶ *
TLC	6.01±1.43		6.36±1.47		0.714
FEV ₁ after terbutaline	2.70±0.73	0.0013*	3.95±0.66	0.057	0.019*
IVC after terbutaline	3.88±0.95	0.51	4.79±0.73	0.035	0.146

*Denote significant difference of $p < 0.05$ using † two tailed paired t test before and after terbutaline treatment and two-tailed paired t test between observed and predicted values.

Plus-minus values are means±SD.

BMI, body mass index; IVC, inspiratory vital capacity; FEV₁, forced expiratory volume in 1 s; FEV₁/FVC, FEV₁%; TLC, total lung capacity.

alleles. These findings provide a direct connection between FGF10 expression and human lung function. Patients with FGF10 insufficiency, also associated with the ALSG syndrome, have a significantly reduced FEV₁ (68% of predicted) and an average FEV₁/IVC of 0.65 (normally above 0.70–0.75) consistent with obstructive lung disease. This is supported by a normal TLC and a disproportionately and slightly reduced IVC (83% of predicted). In COPD, both FEV₁ and IVC are below predicted levels, but the FEV₁/IVC ratio is normal or increased.⁴⁸ The modest increase in FEV₁ (3.7% of predicted value) after terbutaline treatment in our patients indicates that the obstructive symptoms are irreversible. A reversible obstruction (eg, asthma) is defined by an increase of at least 12% in FEV₁ in response to terbutaline treatment.⁴⁹ The degree of COPD in our group of patients is classified as moderate or stage II COPD (FEV₁/IVC <LLN and 60% ≤ FEV₁ <69% of predicted).⁴¹

To validate our results from spirometric measures in patients with FGF10 haploinsufficiency we studied the pulmonary functions in *Fgf10* heterozygous mice. Even though FGF10 deficient mice show a reduction in pulmonary functions similar to those observed in our patients, these results are most probably due to the smaller size of the *Fgf10* +/- mice. Studies of pulmonary functions in mice indicate that direct comparisons between mice and human for key variables such as FEV and FVC are less useful.⁵⁰ The mouse lung shares general structure and physiological mechanisms with the human lung but with some differences. Mice have fewer submucosal glands restricted to the upper trachea and only six to eight branches before reaching the terminal bronchiole.⁵¹ Furthermore, the respiratory bronchioli—structures shown to be involved in the development of emphysema—are absent in the mouse.⁵² These differences may, in part, explain the differences in spirometric measurements between mice and man.

Despite tremendous efforts to understand the genetics behind COPD, most factors remain obscure with rare exceptions such as α 1-antitrypsin deficiency.^{20 43–47 53–56} Our results support the idea that genetic variants affecting the FGF10 signalling pathway are important determinants of lung function that may ultimately contribute to COPD. Specifically, we show that FGF10 haploinsufficiency affects lung function measures, providing a model for a dosage sensitive effect of FGF10 in the development of COPD.

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Competing interests None.

Ethics approval The human studies were approved by the local ethics committee in Jönköping, Sweden, and with the informed consent of the patients. The animal studies were approved by the local ethics committee in Uppsala, Sweden, and performed according to governmental guidelines.

Contributors JK wrote the manuscript, performed statistical analyses and interpreted the data. CB, JB, HFH, and CSB performed the experiments. PB and BB participated in study design and data interpretation. ND designed the study and edited the manuscript.

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REFERENCES

- Raherison C. [Epidemiology of chronic obstructive pulmonary disease] In French. *Presse Med* 2009;**38**:400–5.
- Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, Zielinski J. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007;**176**:532–55.
- Tashkin DP, Altose MD, Connett JE, Kanner RE, Lee WW, Wise RA. Methacholine reactivity predicts changes in lung function over time in smokers with early chronic obstructive pulmonary disease. The Lung Health Study Research Group. *Am J Respir Crit Care Med* 1996;**153**(6 Pt 1):1802–11.
- Molano NA. Genetics of COPD. *Chest* 2004;**125**:1929–40.
- DeMeo DL, Carey VJ, Chapman HA, Reilly JJ, Ginns LC, Speizer FE, Weiss ST, Silverman EK. Familial aggregation of FEF(25-75) and FEF(25-75)/FVC in families with severe, early onset COPD. *Thorax* 2004;**59**:396–400.
- Marciniak SJ, Lomas DA. What can naturally occurring mutations tell us about the pathogenesis of COPD? *Thorax* 2009;**64**:359–64.
- Cardoso WV, Lu J. Regulation of early lung morphogenesis: questions, facts and controversies. *Development* 2006;**133**:1611–24.
- Chen F, Cao Y, Qian J, Shao F, Niederreither K, Cardoso WV. A retinoic acid-dependent network in the foregut controls formation of the mouse lung primordium. *J Clin Invest* 2010;**120**:2040–8.
- McQuilter JL, Yuen K, Williams B, Bertoncello I. Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung. *Proc Natl Acad Sci U S A* 2010;**107**:1414–19.
- Shi W, Chen F, Cardoso WV. Mechanisms of lung development: contribution to adult lung disease and relevance to chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2009;**6**:558–63.
- Crosby LM, Waters CM. Epithelial repair mechanisms in the lung. *Am J Physiol Lung Cell Mol Physiol* 2010;**298**:L715–31.
- Bellucci S, Grindley J, Emoto H, Itoh N, Hogan BL. Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung. *Development* 1997;**124**:4867–78.
- Makarenkova HP, Ito M, Govindarajan V, Faber SC, Sun L, McMahon G, Overbeek PA, Lang RA. FGF10 is an inducer and Pax6 a competence factor for lacrimal gland development. *Development* 2000;**127**:2563–72.
- Ohuchi H, Hori Y, Yamasaki M, Harada H, Sekine K, Kato S, Itoh N. FGF10 acts as a major ligand for FGF receptor 2 IIIb in mouse multi-organ development. *Biochem Biophys Res Commun* 2000;**277**:643–9.
- Li C, Hu L, Xiao J, Chen H, Li JT, Bellucci S, Delanghe S, Minoo P. Wnt5a regulates Shh and Fgf10 signaling during lung development. *Dev Biol* 2005;**287**:86–97.
- Lebeche D, Malpel S, Cardoso WV. Fibroblast growth factor interactions in the developing lung. *Mech Dev* 1999;**86**:125–36.
- Abler LL, Mansour SL, Sun X. Conditional gene inactivation reveals roles for Fgf10 and Fgfr2 in establishing a normal pattern of epithelial branching in the mouse lung. *Dev Dyn* 2009;**238**:1999–2013.
- Desai TJ, Chen F, Lu J, Qian J, Niederreither K, Dolle P, Chambon P, Cardoso WV. Distinct roles for retinoic acid receptors alpha and beta in early lung morphogenesis. *Dev Biol* 2006;**291**:12–24.
- Serra R, Pelton RW, Moses HL. TGF beta 1 inhibits branching morphogenesis and N-myc expression in lung bud organ cultures. *Development* 1994;**120**:2153–61.
- Chuang PT, Kawcak T, McMahon AP. Feedback control of mammalian Hedgehog signaling by the Hedgehog-binding protein, Hip1, modulates Fgf signaling during branching morphogenesis of the lung. *Genes Dev* 2003;**17**:342–7.
- Min H, Danilenko DM, Scully SA, Bolon B, Ring BD, Tarpley JE, DeRose M, Simonet WS. Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless. *Genes Dev* 1998;**12**:3156–61.
- Sekine K, Ohuchi H, Fujiwara M, Yamasaki M, Yoshizawa T, Sato T, Yagishita N, Matsui D, Koga Y, Itoh N, Kato S. Fgf10 is essential for limb and lung formation. *Nat Genet* 1999;**21**:138–41.
- Arman E, Haffner-Krausz R, Chen Y, Heath JK, Lonai P. Targeted disruption of fibroblast growth factor (FGF) receptor 2 suggests a role for FGF signaling in pregastrulation mammalian development. *Proc Natl Acad Sci U S A* 1998;**95**:5082–7.
- De Moerlooze L, Spencer-Dene B, Revest JM, Hajjhosseini M, Rosewell I, Dickson C. An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal-epithelial signalling during mouse organogenesis. *Development* 2000;**127**:483–92.
- Upadhyay D, Panduri V, Kamp DW. Fibroblast growth factor-10 prevents asbestos-induced alveolar epithelial cell apoptosis by a mitogen-activated protein kinase-dependent mechanism. *Am J Respir Cell Mol Biol* 2005;**32**:232–8.
- Gupte VV, Ramasamy SK, Reddy R, Lee J, Weinreb PH, Violette SM, Guenther A, Warburton D, Driscoll B, Minoo P, Bellucci S. Overexpression of fibroblast growth factor-10 during both inflammatory and fibrotic phases attenuates bleomycin-induced pulmonary fibrosis in mice. *Am J Respir Crit Care Med* 2009;**180**:424–36.
- Pogach MS, Cao Y, Millien G, Ramirez MI, Williams MC. Key developmental regulators change during hyperoxia-induced injury and recovery in adult mouse lung. *J Cell Biochem* 2007;**100**:1415–29.
- Entesarian M, Dahlqvist J, Shashi V, Stanley CS, Falahat B, Reardon W, Dahl N. FGF10 missense mutations in aplasia of lacrimal and salivary glands (ALSG). *Eur J Hum Genet* 2007;**15**:379–82.
- Entesarian M, Mattsson H, Klar J, Bergendal B, Olson L, Arakaki R, Hayashi Y, Ohuchi H, Falahat B, Bolstad AI, Jonsson R, Wahren-Herlenius M, Dahl N. Mutations in the gene encoding fibroblast growth factor 10 are associated with aplasia of lacrimal and salivary glands. *Nat Genet* 2005;**37**:125–7.

30. **Milunsky JM**, Zhao G, Maher TA, Colby R, Everman DB. LADD syndrome is caused by FGF10 mutations. *Clin Genet* 2006;**69**:349–54.
31. **Rohmann E**, Brunner HG, Kayserili H, Uyguner O, Nurnberg G, Lew ED, Dobbie A, Eswarakumar VP, Uzumcu A, Ulubil-Emeroglu M, Leroy JG, Li Y, Becker C, Lehnerdt K, Cremers CW, Yuksel-Apak M, Nurnberg P, Kubisch C, Schlessinger J, van Bokhoven H, Wollnik B. Mutations in different components of FGF signaling in LADD syndrome. *Nat Genet* 2006;**38**:414–17.
32. **Francannet C**, Vanlieferinghen P, Dechelotte P, Urbain MF, Campagne D, Malpuech G. LADD syndrome in five members of a three-generation family and prenatal diagnosis. *Genet Couns* 1994;**5**:85–91.
33. **Cardoso WV**, Whitsett JA. Resident cellular components of the lung: developmental aspects. *Proc Am Thorac Soc* 2008;**5**:767–71.
34. **Stanojevic S**, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, Rosenthal M, Corey M, Lebecque P, Cole TJ. Reference ranges for spirometry across all ages: a new approach. *Am J Respir Crit Care Med* 2008;**177**:253–60.
35. **Anon**. Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society. *Am Rev Respir Dis* 1991;**144**:1202–18.
36. **Roberts CM**, MacRae KD, Winning AJ, Adams L, Seed WA. Reference values and prediction equations for normal lung function in a non-smoking white urban population. *Thorax* 1991;**46**:643–50.
37. **Berglund E**, Birath G, Bjure J, Grimby G, Kjellmer I, Sandqvist L, Soderholm B. Spirometric studies in normal subjects. I. Forced expiratory volumes in subjects between 7 and 70 years of age. *Acta Med Scand* 1963;**173**:185–92.
38. **Hedenstrom H**, Malmberg P, Agarwal K. Reference values for lung function tests in females. Regression equations with smoking variables. *Bull Eur Physiopathol Respir* 1985;**21**:551–7.
39. **Hedenstrom H**, Malmberg P, Fridriksson HV. Reference values for lung function tests in men: regression equations with smoking variables. *Ups J Med Sci* 1986;**91**:299–310.
40. **Celli BR**, MacNee W. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J* 2004;**23**:932–46.
41. **Pellegrino R**, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J. Interpretative strategies for lung function tests. *Eur Respir J* 2005;**26**:948–68.
42. **Tiffeneau R**, Pinelli A. Air circulant et air captif dans l'exploration de la fonction ventilatoire pulmonaire. *Paris Med* 1947;**133**:624–8.
43. **Pillai SG**, Ge D, Zhu G, Kong X, Shianna KV, Need AC, Feng S, Hersh CP, Bakke P, Gulsvik A, Ruppert A, Lodrup Carlsen KC, Roses A, Anderson W, Rennard SI, Lomas DA, Silverman EK, Goldstein DB. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. *PLoS Genet* 2009;**5**:e1000421.
44. **Wilk JB**, Chen TH, Gottlieb DJ, Walter RE, Nagle MW, Brandler BJ, Myers RH, Borecki IB, Silverman EK, Weiss ST, O'Connor GT. A genome-wide association study of pulmonary function measures in the Framingham Heart Study. *PLoS Genet* 2009;**5**:e1000429.
45. **Hancock DB**, Eijgelsheim M, Wilk JB, Gharib SA, Loehr LR, Marcicante KD, Franceschini N, van Durme YM, Chen TH, Barr RG, Schabath MB, Couper DJ, Brusselle GG, Psaty BM, van Duijn CM, Rotter JI, Uitterlinden AG, Hofman A, Punjabi NM, Rivadeneira F, Morrison AC, Enright PL, North KE, Heckbert SR, Lumley T, Stricker BH, O'Connor GT, London SJ. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nat Genet* 2010;**42**:45–52.
46. **Repapi E**, Sayers I, Wain LV, Burton PR, Johnson T, Obeidat M, Zhao JH, Ramasamy A, Zhai G, Vitart V, Huffman JE, Igl W, Albrecht E, Deloukas P, Henderson J, Granel R, McArdle WL, Rudnicka AR, Barroso I, Loos RJ, Wareham NJ, Mustelin L, Rantanen T, Surakka I, Imboden M, Wichmann HE, Grkovic I, Jankovic S, Zgaga L, Hartikainen AL, Peltonen L, Gyllensten U, Johansson A, Zaboli G, Campbell H, Wild SH, Wilson JF, Glaser S, Homuth G, Volzke H, Mangino M, Soranzo N, Spector TD, Polasek O, Rudan I, Wright AF, Heliouvaara M, Ripatti S, Pouta A, Naluai AT, Olin AC, Toren K, Cooper MN, James AL, Palmer LJ, Hingorani AD, Wannamethee SG, Whincup PH, Smith GD, Ebrahim S, McKeever TM, Pavord ID, MacLeod AK, Morris AD, Porteous DJ, Cooper C, Dennison E, Shaheen S, Karrasch S, Schnabel E, Schulz H, Grallert H, Bouatia-Naji N, Delplanque J, Froguel P, Blakey JD, Britton JR, Morris RW, Holloway JW, Lawlor DA, Hui J, Nyberg F, Jarvelin MR, Jackson C, Kahonen M, Kaprio J, Probst-Hensch NM, Koch B, Hayward C, Evans DM, Elliott P, Strachan DP, Hall IP, Tobin MD. Genome-wide association study identifies five loci associated with lung function. *Nat Genet* 2010;**42**:36–44.
47. **Qu P**, Roberts J, Li Y, Albrecht M, Cummings OW, Eble JN, Du H, Yan C. Stat3 downstream genes serve as biomarkers in human lung carcinomas and chronic obstructive pulmonary disease. *Lung Cancer* 2009;**63**:341–7.
48. **Aaron SD**, Dales RE, Cardinal P. How accurate is spirometry at predicting restrictive pulmonary impairment? *Chest* 1999;**115**:869–73.
49. **Bateman ED**, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE, Zar HJ. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008;**31**:143–78.
50. **Vanoirbeek JA**, Rinaldi M, De Vooght V, Haenen S, Bobic S, Gayan-Ramirez G, Hoet PH, Verbeke E, Decramer M, Nemery B, Janssens W. Noninvasive and invasive pulmonary function in mouse models of obstructive and restrictive respiratory diseases. *Am J Respir Cell Mol Biol* 2010;**42**:96–104.
51. **Rawlins EL**, Hogan BL. Interstitial growth factor signaling and the development of mouse tracheal submucosal glands. *Dev Dyn* 2005;**233**:1378–85.
52. **Shapiro SD**. Transgenic and gene-targeted mice as models for chronic obstructive pulmonary disease. *Eur Respir J* 2007;**29**:375–8.
53. **Sampsonas F**, Karkoulas K, Kaparianos A, Spiropoulos K. Genetics of chronic obstructive pulmonary disease, beyond a1-antitrypsin deficiency. *Curr Med Chem* 2006;**13**:2857–73.
54. **Hunninghake GM**, Cho MH, Tesfaigzi Y, Soto-Quiros ME, Avila L, Lasky-Su J, Stidley C, Melen E, Soderhall C, Hallberg J, Kull I, Kere J, Svartengren M, Pershagen G, Wickman M, Lange C, Demeo DL, Hersh CP, Klanderman BJ, Raby BA, Sparrow D, Shapiro SD, Silverman EK, Litonjua AA, Weiss ST, Celedon JC. MMP12, lung function, and COPD in high-risk populations. *N Engl J Med* 2009;**361**:2599–608.
55. **van Diemen CC**, Postma DS, Aulchenko YS, et al. Novel strategy to identify genetic risk factors for COPD severity: a genetic isolate. *Eur Respir J* 2010;**35**:768–75.
56. **Cho MH**, Boutaoui N, Klanderman BJ, Sylvia JS, Ziniti JP, Hersh CP, DeMeo DL, Hunninghake GM, Litonjua AA, Sparrow D, Lange C, Won S, Murphy JR, Beaty TH, Regan EA, Make BJ, Hokanson JE, Crapo JD, Kong X, Anderson WH, Tal-Singer R, Lomas DA, Bakke P, Gulsvik A, Pillai SG, Silverman EK. Variants in FAM13A are associated with chronic obstructive pulmonary disease. *Nat Genet* 2010;**42**:200–2.