The role of accelerated ageing in aberrant lung tissue repair and remodelling in COPD

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CHAPTER 1

General Introduction & Scope of this Thesis

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disease that causes severe respiratory symptoms and a poor quality of life. COPD is characterized physiologically by airway obstruction and histologically by chronic inflammatory processes in the lungs that drive disturbed lung tissue remodelling, including emphysema and chronic bronchitis (1-3). Emphysema includes the loss of alveoli and collapse of small airways, functionally this results in impaired gas exchange and breathlessness. Chronic bronchitis causes airflow limitation as a result of inflammation-induced excessive mucus production and airway wall thickening (2-4). Excessive mucus production is also a major contributor to the development of chronic cough, which together with breathlessness affects daily activities. COPD develops slowly and symptoms are apparent later in life. Thereby, COPD is mainly prevalent in the elderly with an age of approximately 65 years or older (5). COPD is diagnosed through clinical assessment in combination with spirometry to measure lung function, whereas the latter also determines the Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages, which classifies the severity of disease (Table 1) (6, 7). Recently, symptoms and risk of exacerbations have been included in grouping the disease severity of patients, called the ABCD assessment (Figure 1) (7).

The major risk factor for the development of COPD is cigarette smoking. Exposure to other noxious gases, including air pollution and occupational exposures can also result in COPD. These exposures cause inflammation that is thought to lead to lung tissue destruction and the thickening of the airway walls. However, not all smokers develop COPD and 25-45% of COPD patients have never smoked (7, 8). Hence, genetic susceptibility plays an essential role in disease pathogenesis. Till now, alpha-1 antitrypsin deficiency is the best known genetic risk factor for emphysema (9), which causes disease in smokers, and only occasionally in non-smokers. Furthermore, genome-wide association studies have found multiple COPD susceptibility genes that were associated with lower lung function and COPD (10-13). However, the exact role of these genes in the pathogenesis of COPD is not fully understood yet.

The WHO estimated a global COPD prevalence of 251 million cases in 2016 and expects an increase in prevalence in the coming years (14). This expected increase in prevalence is caused by higher smoking prevalence in low and middle-income countries and ageing of the population globally. COPD mortality in 2015 has been estimated at 3 million, which is 5% of global deaths and thereby the third leading cause of death (14). Moreover, COPD is the 6th and 5th leading cause of death in 2018 in The Netherlands and Australia respectively. The burden of disease, expressed in Disability Adjusted Life Years, of COPD was ranked 4th and 6th in The Netherlands and Australia respectively. The total healthcare costs of COPD in the EU has been estimated at €141.4 billion, which is an average annual cost of €6,147 per patient (15). In The Netherlands, the number of hospital admissions was 33,735 with on average 7.6 days of admission, and the total healthcare costs were €912 million in

2017 (Dutch National Institute for Public Health and the Environment; RIVM). In Australia, the number of hospital admissions was 77,660 and the total healthcare costs were estimated at $977 million in 2016 (Australian Institute of Health and Welfare; AIHW). Patients with severe COPD with more symptoms represent a significant proportion of hospital admissions and healthcare costs.

Since the pathogenesis of COPD is largely unknown, current treatment strategies are limited and mainly aimed at improving symptoms, without reducing disease progression (7). At the moment the most effective measure to slow down the progression of the disease is preventing exposure to the noxious gases, including cessation of cigarette smoking. E-cigarettes are used as an alternative or cessation device for cigarette smoking, but the effectiveness and safety as cessation aid are largely unknown and controversial (16-18). In addition, an increasing number of studies are being published showing that E-cigarette use is not harmless, including more reports on cases of E-cigarette vaping associated lung injury (EVALI) (19-23). To relieve symptoms, bronchodilators are used that reduce the airflow limitation mainly by relaxation of the airway smooth muscle. Short-acting bronchodilators are used to treat acute bronchoconstriction and long-acting bronchodilators are used to control and prevent symptoms (7). Anti-inflammatory agents are used to treat COPD exacerbations and reduce future risk of exacerbation, which is an acute worsening of the symptoms that are often caused by an infection (7). In patients with severe COPD, oxygen supplementation therapies are used after acute respiratory failure including mechanical ventilation treatments. Novel strategies targeting severe emphysema are various bronchoscopic lung volume reduction methods that reduce breathlessness and improve lung function and quality of life (24, 25). Ultimately, the only therapeutic option left for very severe COPD patients is lung transplantation. So, since no effective treatment options are available to reduce disease progression, new therapeutic targets need to be found. Therefore, novel insights into the pathogenesis of COPD are urgently needed.

Table 1: Classification of COPD GOLD stages based on airflow limitation

GOLD stage	Severity	FEV_1 % predicted
I	Mild	> 80 %
II	Moderate	50 – 80 %
III	Severe	30 – 50 %
IV	Very severe	< 30 %

COPD is being diagnosed when FEV_1/FVC <70%.
FEV_1: Forced expiratory volume in 1 second. Determined by spirometry measurements.
FVC: forced vital capacity. Determined by spirometry measurements.

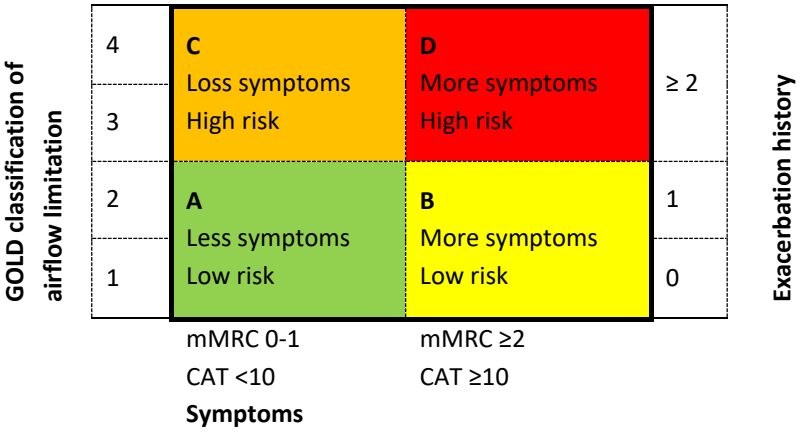

Figure 1: Assessment of COPD severity by use of ABCD disease groups. *Exacerbation risk is estimated by GOLD stages of airflow limitation (left) and exacerbation history (right). Symptoms (bottom) are assessed by modified Medical Research Council dyspnoea score (mMRC) and COPD Assessment Test score (CAT). Figure adapted from Agusti et al. (26) and Vogelmeier et al. (7).*

Normal and accelerated lung ageing

Since life expectancy is increasing worldwide, the interest in the role of ageing in health and disease has increased. As the general population is ageing, the prevalence of chronic and age-related diseases will rise as well. Ageing is described as the progressive decline in normal homeostasis, which leads to an increased risk of diseases and death (27). Recently, nine hallmarks of ageing have been described (Figure 2), which roughly can be divided into three categories; causes of damage, responses to damage and the phenotypic changes (27). During normal lung ageing, lung function declines over time and the alveolar spaces enlarge, called senile emphysema (28-30). Senile emphysema is mainly caused by loss of lung structure and elasticity. The characteristics of senile emphysema are to some extent comparable to pathologic emphysema seen in COPD. However, in COPD, chronic inflammation and tissue damage cause emphysema, including the destruction of alveolar septa and alveolar structure, which is in general maintained in senile emphysema (29). The rate of lung function decline is influenced by genetic factors and environmental exposures, including cigarette smoking (31-35). It should be noted that 4-13% of individuals never reach the maximum average lung function (FEV_1 of 100% predicted), which can increase their risk of COPD development, because of less spare lung capacity (36). Previous studies found multiple similarities between aged lungs and COPD lungs (37, 38). Features of ageing demonstrated in COPD include more inflammation, DNA damage, oxidative stress and cellular senescence, and reduced ability to repair DNA and protein damages (see chapter 2 for a complete review on this topic). Therefore, COPD has been postulated as a disease of accelerated ageing.

Severe, early-onset COPD

With respect to accelerated ageing, a group of patients that is of particular interest are severe, early-onset (SEO-) COPD patients. These patients develop very severe disease (GOLD stage IV, see Table 1) at a relatively young age (age <53, as defined by Silverman *et al.* (39)) with relatively low numbers of pack-years of cigarette smoking compared to the majority of COPD patients that develop symptoms from around 65 years of age (40). Therefore, SEO-COPD patients appear to have a high susceptibility to develop COPD. A large study, called COPDGene, found that 9% of severe (GOLD III-IV) COPD patients were SEO-COPD patients (41). Although the SEO-COPD patients represent a small subgroup of patients, these patients account for a significant proportion of hospital admissions and healthcare costs (42-44). Since SEO-COPD patients develop severe symptoms at a young age, we hypothesize that accelerated ageing may especially play a role in these patients.

Figure 2: *The Hallmarks of ageing. Figure reused with permission from Elsevier (27).*

ECM dysregulation in ageing and COPD

Recently, extracellular matrix (ECM) dysregulation has been proposed as an additional hallmark for lung ageing (38). The ECM is essential for the structure of the lung and tissue repair and remodelling processes. Major components of lung ECM are collagen, elastin, fibronectin and proteoglycans. Upon ageing the ECM changes, with in general an increase in fibrosis and loss of elasticity (45, 46). General COPD-associated ECM changes include increased fibrosis of the airway walls and ECM breakdown and lack of ECM repair in the alveoli, resulting in emphysema (47-50). Recently, a study in our group demonstrated differences in ECM gene expression with ageing in human lung tissue (51). Moreover, pathway analysis of the interaction analysis between age and COPD suggested that age-related changes in ECM, including several collagen genes, were larger in COPD patients as compared to non-COPD controls. Lung fibroblasts are the major producers of ECM and regulate ECM homeostasis and therefore play an important role in lung repair and remodelling processes. In COPD, the repair functions are impaired and remodelling processes are altered leading to the loss of alveoli and fibrosis around the airways. Airway smooth muscle cells are another cell type that has a role in tissue repair and remodelling in COPD. In COPD the ASM mass is increased, accompanied with enhanced ECM deposition (52). Alterations in ECM regulation have been demonstrated in COPD lungs previously, including higher collagen and versican production, and lower elastin, decorin and perlecan production. (48, 53-56). In addition, *in vitro* treatment with cigarette smoke extract (CSE) and TGF-β resulted in altered ECM production, including increased ECM protein production (53, 57, 58), where TGF-β levels are higher in COPD lungs and therefore often used to mimic *in vivo* COPD conditions. The role of accelerated ageing in aberrant lung tissue repair and remodelling in COPD and age-related changes in lung fibroblasts remains to be elucidated.

SCOPE OF THIS THESIS

We hypothesize that accelerated ageing is involved in the pathogenesis of COPD by affecting lung tissue repair and remodelling processes (Figure 3). Therefore, the overall aim of this thesis is to elucidate the role of accelerated ageing in aberrant tissue repair and remodelling in COPD.

Firstly, **chapter 2** gives an overview of the evidence available at the start of this project on the role of ageing in lung tissue repair and remodelling in COPD. It describes the similarities between lung ageing and COPD in more detail and gives a comprehensive overview of all data from published studies that demonstrated ageing hallmarks in lung tissue and structural cells from COPD patients or *in vitro* cultured primary structural lung cells treated with cigarette smoke extract (CSE). Finally, this review describes the gap in the scientific knowledge regarding the role of *accelerated* ageing in tissue repair and remodelling in COPD, which formed the basis for the experiments described in this thesis.

In the first experimental chapter (**chapter 3**) of this thesis we measured differential gene and miRNA expression with increasing age in biopsies from healthy individuals to assess key genes and regulators (miRNA's) involved in normal lung ageing. In **chapter 4**, we assessed whether primary parenchymal lung fibroblasts from COPD and SEO-COPD patients have features of accelerated ageing compared to fibroblasts from non-COPD controls by analysing multiple ageing hallmarks in these cells. In addition, we assessed whether accelerated ageing has functional consequences on ECM regulation of the fibroblasts. Following on our results in chapter 4, we aimed to define the senescence-associated secretory phenotype (SASP) of primary parenchymal lung fibroblasts in **chapter 5** and assessed whether these SASP proteins were secreted in higher levels by COPD-derived fibroblasts compared to non-COPD control-derived fibroblasts. Since we found a link between cellular senescence and ECM regulation in COPD-derived fibroblasts, and we know that ASMCs play a role in ECM regulation as well, we assessed in **chapter 6** whether COPD-derived ASMCs also have higher levels of cellular senescence compared to ASMCs from non-COPD controls and whether this is linked with ECM regulation. In the last experimental chapter (**chapter 7**), we assessed whether E-cigarette vapour exposure, similar to CSE, induces cellular senescence in primary parenchymal lung fibroblasts and whether this affects the repair function of these fibroblasts. Finally, in **chapter 8** we summarize all findings from this thesis, discuss the relevance and implications of these findings, and describe some of the future perspectives in the field of accelerated ageing and lung tissue repair and remodelling.

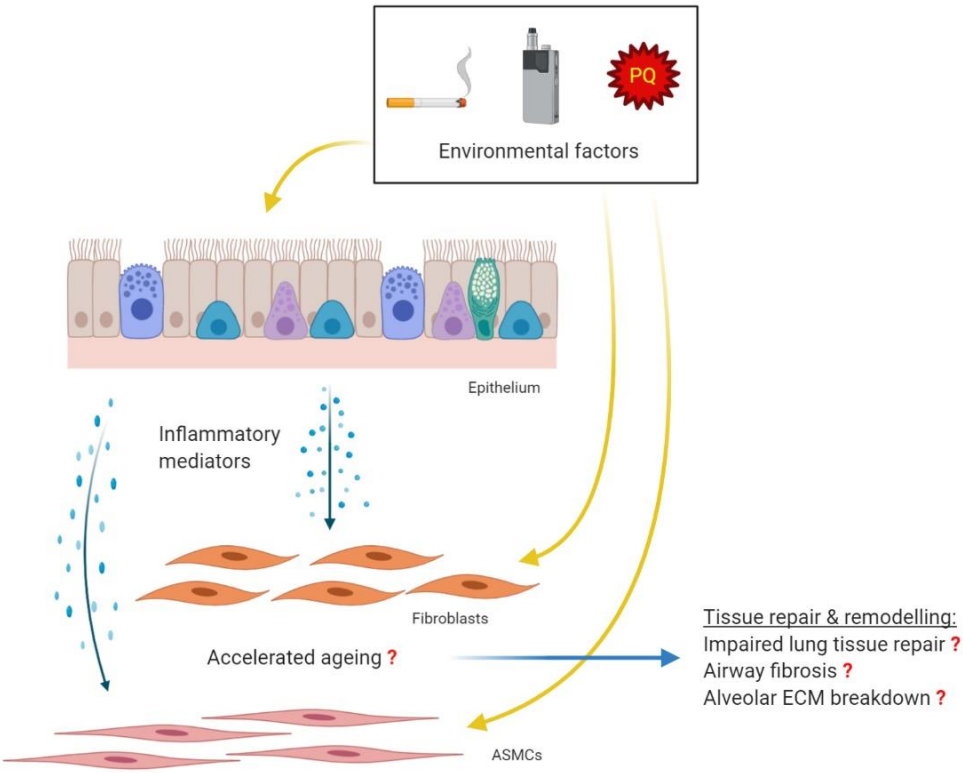

Figure 3: Hypothesized role of accelerated ageing in tissue repair and remodelling in COPD. *We hypothesize that lung fibroblasts and airway smooth muscle cells (ASMCs) from COPD patients have an accelerated ageing phenotype. We propose that this phenotype can be caused by environmental factors, including cigarette smoke, E-cigarette vapour, and Paraquat (PQ), which is another COPD risk factor by occupational exposure, directly or via the effect on the epithelium in combination with an impairment in age-related repair and maintenance mechanisms. In addition, we hypothesize that this ageing phenotype affects tissue repair and remodelling with impaired lung tissue repair, induced airway fibrosis and alveolar ECM breakdown. Created with BioRender.com.*

REFERENCES

(1) Barnes PJ. Chronic obstructive pulmonary disease. N Engl J Med 2000;343:269-280.
(2) Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, Pare PD. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med 2004;350:2645-2653.
(3) Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. Annu Rev Pathol 2009;4:435-459.
(4) Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic obstructive lung disease. N Engl J Med 1968;278:1355-1360.
(5) Raherison C, Girodet PO. Epidemiology of COPD. Eur Respir Rev 2009;18:213-221.
(6) Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, Stockley RA, Sin DD, Rodriguez-Roisin R. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013;187:347-365.
(7) Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Chen R, Decramer M, Fabbri LM, Frith P, Halpin DM, Lopez Varela MV, Nishimura M, Roche N, Rodriguez-Roisin R, Sin DD, Singh D, Stockley R, Vestbo J, Wedzicha JA, Agusti A. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary. Am J Respir Crit Care Med 2017;195:557-582.
(8) Salvi SS, Barnes PJ. Chronic obstructive pulmonary disease in non-smokers. Lancet 2009;374:733-743.
(9) Silverman EK, Spira A, Pare PD. Genetics and genomics of chronic obstructive pulmonary disease. Proc Am Thorac Soc 2009;6:539-542.
(10) Soler Artigas M, Loth DW, Wain LV, Gharib SA, Obeidat M, Tang W, Zhai G, Zhao JH, Smith AV, Huffman JE, Albrecht E, Jackson CM, Evans DM, Cadby G, Fornage M, Manichaikul A, Lopez LM, Johnson T, Aldrich MC, Aspelund T, Barroso I, Campbell H, Cassano PA, Couper DJ, Eiriksdottir G, Franceschini N, Garcia M, Gieger C, Gislason GK, Grkovic I, Hammond CJ, Hancock DB, Harris TB, Ramasamy A, Heckbert SR, Heliovaara M, Homuth G, Hysi PG, James AL, Jankovic S, Joubert BR, Karrasch S, Klopp N, Koch B, Kritchevsky SB, Launer LJ, Liu Y, Loehr LR, Lohman K, Loos RJ, Lumley T, Al Balushi KA, Ang WQ, Barr RG, Beilby J, Blakey JD, Boban M, Boraska V, Brisman J, Britton JR, Brusselle GG, Cooper C, Curjuric I, Dahgam S, Deary IJ, Ebrahim S, Eijgelsheim M, Francks C, Gaysina D, Granell R, Gu X, Hankinson JL, Hardy R, Harris SE, Henderson J, Henry A, Hingorani AD, Hofman A, Holt PG, Hui J, Hunter ML, Imboden M, Jameson KA, Kerr SM, Kolcic I, Kronenberg F, Liu JZ, Marchini J, McKeever T, Morris AD, Olin AC, Porteous DJ, Postma DS, Rich SS, Ring SM, Rivadeneira F, Rochat T, Sayer AA, Sayers I, Sly PD, Smith GD, Sood A, Starr JM, Uitterlinden AG, Vonk JM, Wannamethee SG, Whincup PH, Wijmenga C, Williams OD, Wong A, Mangino M, Marciante KD, McArdle WL, Meibohm B, Morrison AC, North KE, Omenaas E, Palmer LJ, Pietilainen KH, Pin I, Pola Sbreve Ek O, Pouta A, Psaty BM, Hartikainen AL, Rantanen T, Ripatti S, Rotter JI, Rudan I, Rudnicka AR, Schulz H, Shin SY, Spector TD, Surakka I, Vitart V, Volzke H, Wareham NJ, Warrington NM, Wichmann HE, Wild SH, Wilk JB, Wjst M, Wright AF, Zgaga L, Zemunik T, Pennell CE, Nyberg F, Kuh D, Holloway JW, Boezen HM, Lawlor DA, Morris RW, Probst-Hensch N, International Lung Cancer Consortium, GIANT consortium, Kaprio J, Wilson JF, Hayward C, Kahonen M, Heinrich J, Musk AW, Jarvis DL, Glaser S, Jarvelin MR, Ch Stricker BH, Elliott P, O'Connor GT, Strachan DP, London SJ, Hall IP, Gudnason V, Tobin MD. Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. Nat Genet 2011;43:1082-1090.
(11) Decramer M, Janssens W, Miravitlles M. Chronic obstructive pulmonary disease. Lancet 2012;379:1341-1351.
(12) Foreman MG, Campos M, Celedon JC. Genes and chronic obstructive pulmonary disease. Med Clin North Am 2012;96:699-711.
(13) Hobbs BD, de Jong K, Lamontagne M, Bosse Y, Shrine N, Artigas MS, Wain LV, Hall IP, Jackson VE, Wyss AB, London SJ, North KE, Franceschini N, Strachan DP, Beaty TH, Hokanson JE, Crapo JD, Castaldi PJ, Chase RP, Bartz TM, Heckbert SR, Psaty BM, Gharib SA, Zanen P, Lammers JW, Oudkerk M, Groen HJ, Locantore N, Tal-Singer R, Rennard SI, Vestbo J, Timens W, Pare PD, Latourelle JC, Dupuis J, O'Connor GT, Wilk JB, Kim WJ, Lee MK, Oh YM, Vonk JM, de Koning HJ, Leng S, Belinsky SA, Tesfaigzi Y, Manichaikul A, Wang XQ, Rich SS, Barr RG, Sparrow D, Litonjua AA, Bakke P, Gulsvik A, Lahousse L, Brusselle GG, Stricker BH, Uitterlinden AG, Ampleford EJ, Bleecker ER, Woodruff PG, Meyers DA, Qiao D, Lomas DA, Yim JJ, Kim DK, Hawrylkiewicz I, Sliwinski P, Hardin M, Fingerlin TE, Schwartz DA, Postma DS, MacNee W, Tobin MD, Silverman EK, Boezen HM, Cho MH, COPDGene Investigators, ECLIPSE Investigators, LifeLines Investigators, SPIROMICS Research Group, International COPD Genetics Network Investigators, UK BiLEVE Investigators, International COPD Genetics Consortium. Genetic loci associated with

chronic obstructive pulmonary disease overlap with loci for lung function and pulmonary fibrosis. Nat Genet 2017;49:426-432.
(14) World Health Organization (WHO) Fact sheet: Chronic obstructive pulmonary disease (COPD). World Health Organization 2017.
(15) Gibson GJ, Loddenkemper R, Lundbäck B, Sibille Y. European lung white book, Chapter: The economic burden of lung disease. 2013.
(16) Bals R, Boyd J, Esposito S, Foronjy R, Hiemstra PS, Jimenez-Ruiz CA, Katsaounou P, Lindberg A, Metz C, Schober W, Spira A, Blasi F. Electronic cigarettes: a task force report from the European Respiratory Society. Eur Respir J 2019;53:10.1183/13993003.01151-2018. Print 2019 Feb.
(17) Bush A, Bhatt JM, Carroll W, Child F, Connett G, Doull I, Gilchrist F, Grigg J, Langton-Hewer S, Legg J, Lenney W, Paton J, Shields M, Sinha I. The ERS approach to e-cigarettes is entirely rational. Eur Respir J 2020;55:10.1183/13993003.00413-2020. Print 2020 May.
(18) Bozier J, Chivers EK, Chapman DG, Larcombe AN, Bastian NA, Masso-Silva JA, Byun MK, McDonald CF, Crotty Alexander LE, Ween MP. The Evolving Landscape of e-Cigarettes: A Systematic Review of Recent Evidence. Chest 2020;157:1362-1390.
(19) Kalininskiy A, Bach CT, Nacca NE, Ginsberg G, Marraffa J, Navarette KA, McGraw MD, Croft DP. E-cigarette, or vaping, product use associated lung injury (EVALI): case series and diagnostic approach. Lancet Respir Med 2019;7:1017-1026.
(20) Chatham-Stephens K, Roguski K, Jang Y, Cho P, Jatlaoui TC, Kabbani S, Glidden E, Ussery EN, Trivers KF, Evans ME, King BA, Rose DA, Jones CM, Baldwin G, Delaney LJ, Briss P, Ritchey MD, Lung Injury Response Epidemiology/Surveillance Task Force, Lung Injury Response Clinical Task Force. Characteristics of Hospitalized and Nonhospitalized Patients in a Nationwide Outbreak of E-cigarette, or Vaping, Product Use-Associated Lung Injury - United States, November 2019. MMWR Morb Mortal Wkly Rep 2019;68:1076-1080.
(21) Perrine CG, Pickens CM, Boehmer TK, King BA, Jones CM, DeSisto CL, Duca LM, Lekiachvili A, Kenemer B, Shamout M, Landen MG, Lynfield R, Ghinai I, Heinzerling A, Lewis N, Pray IW, Tanz LJ, Patel A, Briss PA, Lung Injury Response Epidemiology/Surveillance Group. Characteristics of a Multistate Outbreak of Lung Injury Associated with E-cigarette Use, or Vaping - United States, 2019. MMWR Morb Mortal Wkly Rep 2019;68:860-864.
(22) Werner AK, Koumans EH, Chatham-Stephens K, Salvatore PP, Armatas C, Byers P, Clark CR, Ghinai I, Holzbauer SM, Navarette KA, Danielson ML, Ellington S, Moritz ED, Petersen EE, Kiernan EA, Baldwin GT, Briss P, Jones CM, King BA, Krishnasamy V, Rose DA, Reagan-Steiner S, Lung Injury Response Mortality Working Group. Hospitalizations and Deaths Associated with EVALI. N Engl J Med 2020;382:1589-1598.
(23) Krishnasamy VP, Hallowell BD, Ko JY, Board A, Hartnett KP, Salvatore PP, Danielson M, Kite-Powell A, Twentyman E, Kim L, Cyrus A, Wallace M, Melstrom P, Haag B, King BA, Briss P, Jones CM, Pollack LA, Ellington S, Lung Injury Response Epidemiology/Surveillance Task Force. Update: Characteristics of a Nationwide Outbreak of E-cigarette, or Vaping, Product Use-Associated Lung Injury - United States, August 2019-January 2020. MMWR Morb Mortal Wkly Rep 2020;69:90-94.
(24) Shah PL, Herth FJ, van Geffen WH, Deslee G, Slebos DJ. Lung volume reduction for emphysema. Lancet Respir Med 2017;5:147-156.
(25) van Geffen WH, Slebos DJ, Herth FJ, Kemp SV, Weder W, Shah PL. Surgical and endoscopic interventions that reduce lung volume for emphysema: a systemic review and meta-analysis. Lancet Respir Med 2019;7:313-324.
(26) Agusti A, Hurd S, Jones P, Fabbri LM, Martinez F, Vogelmeier C, Vestbo J, Rodriguez-Roisin R. FAQs about the GOLD 2011 assessment proposal of COPD: a comparative analysis of four different cohorts. Eur Respir J 2013;42:1391-1401.
(27) Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013;153:1194-1217.
(28) D'Errico A, Scarani P, Colosimo E, Spina M, Grigioni WF, Mancini AM. Changes in the alveolar connective tissue of the ageing lung. An immunohistochemical study. Virchows Arch A Pathol Anat Histopathol 1989;415:137-144.
(29) Verbeken EK, Cauberghs M, Mertens I, Clement J, Lauweryns JM, Van de Woestijne KP. The senile lung. Comparison with normal and emphysematous lungs. 1. Structural aspects. Chest 1992;101:793-799.
(30) Miller MR. Structural and physiological age-associated changes in aging lungs. Semin Respir Crit Care Med 2010;31:521-527.

(31) Celli BR, Thomas NE, Anderson JA, Ferguson GT, Jenkins CR, Jones PW, Vestbo J, Knobil K, Yates JC, Calverley PM. Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: results from the TORCH study. Am J Respir Crit Care Med 2008;178:332-338.
(32) Vestbo J, Edwards LD, Scanlon PD, Yates JC, Agusti A, Bakke P, Calverley PM, Celli B, Coxson HO, Crim C, Lomas DA, MacNee W, Miller BE, Silverman EK, Tal-Singer R, Wouters E, Rennard SI, ECLIPSE Investigators. Changes in forced expiratory volume in 1 second over time in COPD. N Engl J Med 2011;365:1184-1192.
(33) Busch R, Cho MH, Silverman EK. Progress in disease progression genetics: dissecting the genetic origins of lung function decline in COPD. Thorax 2017;72:389-390.
(34) Imboden M, Wielscher M, Rezwan FI, Amaral AFS, Schaffner E, Jeong A, Beckmeyer-Borowko A, Harris SE, Starr JM, Deary IJ, Flexeder C, Waldenberger M, Peters A, Schulz H, Chen S, Sunny SK, Karmaus WJJ, Jiang Y, Erhart G, Kronenberg F, Arathimos R, Sharp GC, Henderson AJ, Fu Y, Piirila P, Pietilainen KH, Ollikainen M, Johansson A, Gyllensten U, de Vries M, van der Plaat DA, de Jong K, Boezen HM, Hall IP, Tobin MD, Jarvelin MR, Holloway JW, Jarvis D, Probst-Hensch NM. Epigenome-wide association study of lung function level and its change. Eur Respir J 2019;54:10.1183/13993003.00457-2019. Print 2019 Jul.
(35) Agusti A, Hogg JC. Update on the Pathogenesis of Chronic Obstructive Pulmonary Disease. N Engl J Med 2019;381:1248-1256.
(36) Agusti A, Noell G, Brugada J, Faner R. Lung function in early adulthood and health in later life: a transgenerational cohort analysis. Lancet Respir Med 2017;5:935-945.
(37) Ito K, Barnes PJ. COPD as a disease of accelerated lung aging. Chest 2009;135:173-180.
(38) Meiners S, Eickelberg O, Konigshoff M. Hallmarks of the ageing lung. Eur Respir J 2015;45:807-827.
(39) Silverman EK, Weiss ST, Drazen JM, Chapman HA, Carey V, Campbell EJ, Denish P, Silverman RA, Celedon JC, Reilly JJ, Ginns LC, Speizer FE. Gender-related differences in severe, early-onset chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000;162:2152-2158.
(40) Hersh CP, DeMeo DL, Al-Ansari E, Carey VJ, Reilly JJ, Ginns LC, Silverman EK. Predictors of survival in severe, early onset COPD. Chest 2004;126:1443-1451.
(41) Foreman MG, Zhang L, Murphy J, Hansel NN, Make B, Hokanson JE, Washko G, Regan EA, Crapo JD, Silverman EK, DeMeo DL, COPDGene Investigators. Early-onset chronic obstructive pulmonary disease is associated with female sex, maternal factors, and African American race in the COPDGene Study. Am J Respir Crit Care Med 2011;184:414-420.
(42) Mullerova H, Maselli DJ, Locantore N, Vestbo J, Hurst JR, Wedzicha JA, Bakke P, Agusti A, Anzueto A. Hospitalized exacerbations of COPD: risk factors and outcomes in the ECLIPSE cohort. Chest 2015;147:999-1007.
(43) Papi A, Magnoni MS, Muzzio CC, Benso G, Rizzi A. Phenomenology of COPD: interpreting phenotypes with the ECLIPSE study. Monaldi Arch Chest Dis 2016;83:721.
(44) Zhu L, Ni Z, Luo X, Zhang Z, Wang S, Meng Z, Gu X, Wang X. The outcome and the influencing factors of the age of onset in post-mortem of chronic bronchitis patients: a retrospective study. Int J Chron Obstruct Pulmon Dis 2018;13:645-652.
(45) Meschiari CA, Ero OK, Pan H, Finkel T, Lindsey ML. The impact of aging on cardiac extracellular matrix. Geroscience 2017;39:7-18.
(46) Birch HL. Extracellular Matrix and Ageing. Subcell Biochem 2018;90:169-190.
(47) Postma DS, Timens W. Remodeling in asthma and chronic obstructive pulmonary disease. Proc Am Thorac Soc 2006;3:434-439.
(48) Annoni R, Lancas T, Yukimatsu Tanigawa R, de Medeiros Matsushita M, de Morais Fernezlian S, Bruno A, Fernando Ferraz da Silva L, Roughley PJ, Battaglia S, Dolhnikoff M, Hiemstra PS, Sterk PJ, Rabe KF, Mauad T. Extracellular matrix composition in COPD. Eur Respir J 2012;40:1362-1373.
(49) Bidan CM, Veldsink AC, Meurs H, Gosens R. Airway and Extracellular Matrix Mechanics in COPD. Front Physiol 2015;6:346.
(50) Burgstaller G, Oehrle B, Gerckens M, White ES, Schiller HB, Eickelberg O. The instructive extracellular matrix of the lung: basic composition and alterations in chronic lung disease. Eur Respir J 2017;50:10.1183/13993003.01805-2016. Print 2017 Jul.
(51) de Vries M, Faiz A, Woldhuis RR, Postma DS, de Jong TV, Sin DD, Bosse Y, Nickle DC, Guryev V, Timens W, van den Berge M, Brandsma CA. Lung tissue gene-expression signature for the ageing lung in COPD. Thorax 2017.

(52) Chung KF. The role of airway smooth muscle in the pathogenesis of airway wall remodeling in chronic obstructive pulmonary disease. Proc Am Thorac Soc 2005;2:347-54; discussion 371-2.
(53) Zandvoort A, Postma DS, Jonker MR, Noordhoek JA, Vos JT, van der Geld YM, Timens W. Altered expression of the Smad signalling pathway: implications for COPD pathogenesis. Eur Respir J 2006;28:533-541.
(54) Hallgren O, Nihlberg K, Dahlback M, Bjermer L, Eriksson LT, Erjefalt JS, Lofdahl CG, Westergren-Thorsson G. Altered fibroblast proteoglycan production in COPD. Respir Res 2010;11:55-9921-11-55.
(55) Larsson-Callerfelt AK, Hallgren O, Andersson-Sjoland A, Thiman L, Bjorklund J, Kron J, Nihlberg K, Bjermer L, Lofdahl CG, Westergren-Thorsson G. Defective alterations in the collagen network to prostacyclin in COPD lung fibroblasts. Respir Res 2013;14:21-9921-14-21.
(56) Eurlings IM, Dentener MA, Cleutjens JP, Peutz CJ, Rohde GG, Wouters EF, Reynaert NL. Similar matrix alterations in alveolar and small airway walls of COPD patients. BMC Pulm Med 2014;14:90-2466-14-90.
(57) Zandvoort A, Postma DS, Jonker MR, Noordhoek JA, Vos JT, Timens W. Smad gene expression in pulmonary fibroblasts: indications for defective ECM repair in COPD. Respir Res 2008;9:83-9921-9-83.
(58) Chen L, Ge Q, Tjin G, Alkhouri H, Deng L, Brandsma CA, Adcock I, Timens W, Postma D, Burgess JK, Black JL, Oliver BG. Effects of cigarette smoke extract on human airway smooth muscle cells in COPD. Eur Respir J 2014;44:634-646.