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Animal models: An essential tool to dissect the heterogeneity of chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease characterized by persistent respiratory symptoms and progressive airflow obstruction. Its main pathophysiology includes chronic airway inflammation, mucus hypersecretion, airway remodeling, and emphysema.[1] Environment-gene-immune interactions drive the occurrence and development of COPD (Figure 1). Environmental and genetic factors may affect baseline lung function, causing abnormal lung development and disrupting lung homeostasis by activating type 1 and type 3 immunity.[2] COPD is predicted to be the third leading cause of death by 2030, and its prevalence continues to increase.[3] Mechanisms underlying the development and progression of COPD and its potential targets remain to be explored.

ANIMAL MODELS ON THE WAY TO DISSECT HETEROGENEITY

Human studies are complicated by individual genetic backgrounds, environmental factors, and limitations in sample collection. Animal models are valuable tools for therapeutic testing before progression to humans (Table 1). Several animal species have been used as a model of COPD, including mice, guinea pigs, rats, hamsters, ferrets, and monkeys. Each animal model exhibits advantages and disadvantages. Mice have become the most popular model owing to their low cost, availability of molecular reagents, mature gene editing, and sequencing technologies.[4] Guinea pigs are superior to other animals in the development of mucus hypersecretion and emphysema;[5] however, there are interspecies differences in lung anatomy, lung development, and maturity. Progressive narrowing of terminal bronchioles accompanied by emphysema typically starts in the respiratory bronchioles, whereas rodents often lack these and have far fewer bronchial branches than humans. Guinea pigs and monkeys have well-developed alveoli at birth, whereas those of rats and mice develop after birth[6] Therefore, it is critical to recognize the limitations of current animal models.

RISK FACTORS MIMIC THE REAL WORLD

Cigarette smoking (CS) is a major risk factor for COPD in high-income countries, whereas exposure to cooking biomass fuels is a major precipitant in low-income countries.[7] CS was the most common COPD-inducer used in previous studies. A variety of animals have been used to establish COPD models with CS, wherein guinea pigs are perhaps the most susceptible species owing to their significant airspace enlargement.[8] Rats appear to be the most resistant to emphysema and develop nonspecific particle overload effects, whereas susceptibility in mice appears to be strain-dependent. Similar to that in humans,

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Access this article online

Website: www.intern-med.com

DOI: 10.2478/jtim-2023-0007

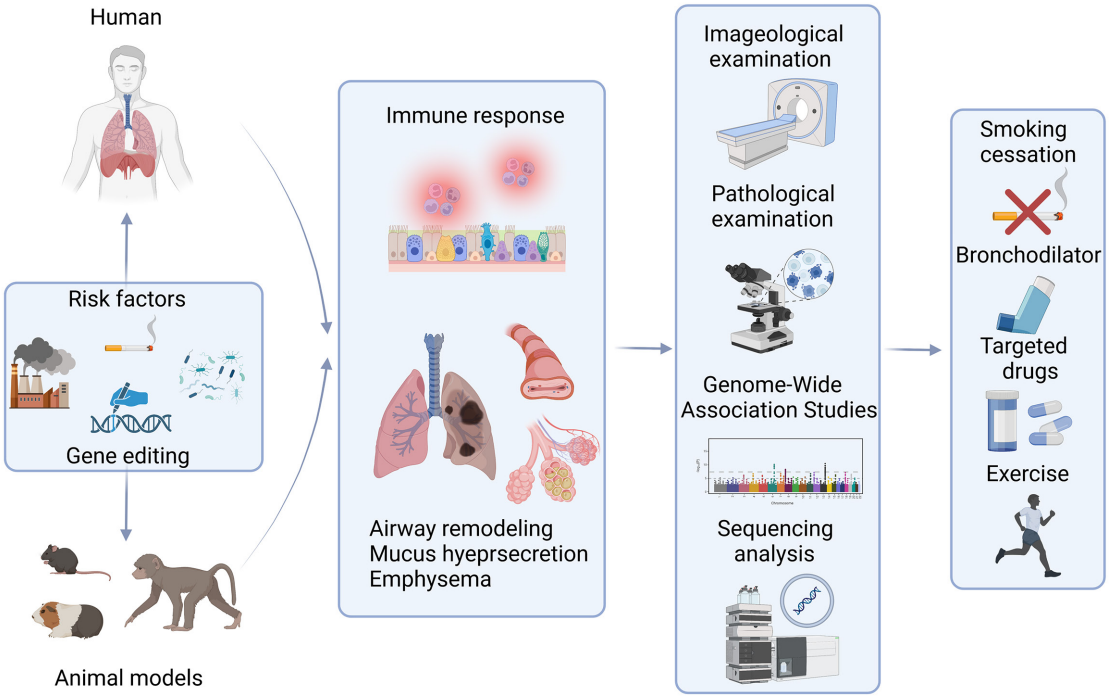

Figure 1. Environment-gene-immune interaction drives the occurrence and development of COPD, wherein combined risk factors induce the novel gene editing animals, and molecular biology and imaging tools are used to explore potential targets for diagnosis and treatment of the disease.

COPD features are not suppressed by corticosteroid treatment in animal models. However, the method of smoke generation, constituents of CS, delivery systems used, and dosage varied among studies, leading to poor homogeneity of results.[9] Pathological phenotypes, such as emphysema, airway remodeling, and pulmonary hypertension, can only be induced in chronic CS models, as it is time-consuming. One month after CS exposure, an acute phase dominated with maximal neutrophil infiltration during the first week, followed by a progressive chronic phase consisting of infiltrating neutrophils, macrophages, and lymphocytes in the lungs.[10] Another limitation is the degree of induced COPD equivalent to Global Initiative for Obstructive Lung Disease (GOLD) stage 1 or 2 in CS models, whereas most exacerbations and deaths occur during GOLD stage 3 or 4, and inflammation persists after smoking cessation in patients with COPD.[11] Therefore, the CS model differs from the real COPD phenotype to some extent.

Lipopolysaccharide (LPS) is present in CS, air pollutants, and organic dust as a major component of the outer cell wall of gram-negative bacteria. Chronic exposure of animals to LPS has been shown to induce pathological features of COPD, including pronounced neutrophilic inflammation, enlarged air spaces, airway remodeling, and decreased lung function.[12, 13] However, robust inflammation can be inhibited by glucocorticoids, and repeated administration induces immune tolerance, which can alleviate neutrophilic inflammation.[14]

Excessive elastase secretion can induce matrix destruction, and the elastase model can induce tissue damage and emphysema. The development of emphysema following the instillation of elastases, including neutrophil elastase (NE), porcine pancreatic elastase (PPE), and papain, results in rapid and significant airspace enlargement, followed by acute neutrophil and macrophage accumulation and mucus cell metaplasia.[15] COPD can be induced by a single instillation and its severity is adjusted to the dosage of elastase,[16] which makes it cheaper and easier to induce than chronic smoke exposure. This model is ideal for testing the efficacy of therapeutic agents, particularly those with the capacity to reverse or repair emphysematous damage to the lungs.[17] However, detailed mechanisms of the COPD proteolytic process are quite different from those of the elastase model. In addition, inflammation is transient and resolves within one week of elastase administration.

Pollutants (PM2.5), ozone, and other irritants were also applied to the experimental animals. Ozone initiates intracellular oxidative stress through the formation of ozonide and hydrogen peroxide, which induces airway inflammation, airway hyperresponsiveness, lung destruction, and a steroid-insensitive phenotype within six weeks.[18] Combination inducers, such as CS, PPE,

Table 1: Different methods of inducing COPD animal models

Stimulant	Species	Methods	Ref.
CS	Mice	Acute model: 5 cigarettes (with filter); for 20 min	[30-32]
		Chronic model: 3 cigarettes per day; 5 days per week; for 7 months	
		Nose only: 75 min per time; twice per day; 5 days per week; for 8 or 12 weeks	
		Whole-body exposure: 5 cigarettes; 4 times per day; 30 min per interval; 5 days per week; for 4 or 24 weeks	
		Nose only: 12 cigarettes per run; twice per day; 5 days per week; for 8 weeks	
		Whole-body exposure: 5 cigarettes (without filter); 4 times per day; 5 days per week; for 4 weeks	
	Rat	7 h per day; 5 days per week; for 7 or 13 months	[33-35]
		1 mL PBS per cigarette; injected intraperitoneally on days 1, 8, and 15	
		5 cigarettes; 30 min per time; twice per day; 6 days per week; for 4 weeks	
	Guinea pig	10 cigarettes per day; 5 days per week; for 1, 3, and 6 months	[36-38]
		7 cigarettes per day; 5 days per week; for 3, 4, and 6 months	
		Nose noly: 7 cigarettes per day (without filter); 5 days per week; for 12 weeks	
LPS	Mice	5 μg LPS (it); twice per week; for 12 weeks	[12, 13]
		2 mg nebulized LPS; 15 min per day; 5 days per week; for 1, 4, and 8 weeks	
	Rat	200 μg LPS (it); twice per week; for 3 weeks	[39]
	Guinea pig	Single exposure: 30 μg/mL nebulized LPS, for 1 h	[14, 40]
		Chronic exposure: 30 μg/mL nebulized LPS, for 1 h, 47 h per interval; for 9 times	
		30 μg/mL nebulized LPS, for 1 h, 47 h per interval; for 15 times	
Elastase	Mice	100 U/kg PPE (it)	[41, 42]
		100 U/kg PPE (it)	
	Rat	75 U PPE (it)	[17, 43]
		25 U/kg PPE, on days 1, and 10	
	Hamster	40 U PPE	[44]
Ozone	Mice	2.5 ppm ozone; 3 h per day; twice per week; for over 6 weeks	[18]
PM2.5	Mice	10 h per day; 7 days per week; for 3 months	[45]
	Rat	2 h per time; twice per day; 5 days per week; for 7 months	[46]

COPD: chronic obstructive pulmonary disease; CS: cigarette smoking; LPS: lipopolysaccharide. PPE: porcine pancreatic elastase; it: intratracheal; ppm: parts per million.

LPS, bacteria, viruses, and pollutants, are widely used to simulate the different stages of COPD, which is of great value in exploring how environmental risk factors affect the development and deterioration of COPD.[19,20] For example, mice were intranasally injected with PPE and LPS for 4 weeks, which can induce COPD-like lung inflammation and alveolar enlargement.[21]

However, the existing single or combined risk factor-induced models can only partially mimic the characteristics of COPD, and more risk factors should be considered to refine the animal models.

GENETIC FACTORS REVEAL SUSCEPTIBILITY

CS is a major risk factor for COPD; however, only 15%–20% of smokers are susceptible to developing COPD and 25%–45% of patients with COPD have never smoked. Therefore, it is being increasingly recognized that COPD

Table 2: Candidate genes for COPD suggested by genome-wide association studies

Gene	Locus	rsID	Function	Phenotype	Ref.
FAM13A	4q22.1	rs2013701 rs7671167 rs10007590 rs2869966 rs2869967 rs2045517	Rho GTPase signaling Fatty acid oxidation Mitochondrial function Alveolar epithelial cells repair and regeneration	Severe COPD Lung function (FEV_1/FVC) Emphysema	[23, 25, 47, 48]
TGFB2	1q41	rs1690789 rs4846480 rs6684205	Lung development Tissue repair and remodeling	Severe COPD Lung function (FEV_1/FVC) Emphysema	[23, 49]
HHIP	4q31.21	rs1828591 rs13118928 rs6537296 rs1542725 rs6817273 rs10519717 rs1980057 rs1032295	Hedgehog signaling pathway Embryonic development Lung organogenesis Oxidative stress alleviation Airway remodeling repression	Severe COPD COPD exacerbation Lung function (FEV_1/FVC, FEV_1) Airflow obstruction Emphysema Fat-free body mass Body mass index	[23, 25, 48, 50-52]
HTR4	5q32	rs7733088 rs11168048 rs7735184	A major neurotransmitter in the CNS Learning, memory, depression, anxiety and feeding behaviour Lung development	Lung function (FEV_1/FVC, FEV_1) Airflow obstruction	[48, 51, 53]
CHRNA3/5	15q25.1	rs8034191 rs1051730 rs17486278 rs16969968 rs8040863 rs55853698 rs6495308 rs13141641	Nicotinic acetylcholine receptor Cell-cycle regulation	Severe COPD Lung function (FEV_1/FVC, FEV_1) Airflow obstruction Emphysema Nicotine addiction	[23, 25, 50, 51]
MMP12	11q22	rs2276109 rs652438 rs626750	Tissue repair and remodelling Protease - antiprotease imbalance	Severe/very severe COPD Lung function (FEV_1) Emphysema	[23, 54]
AGER	6p21.32	rs2070600	Epithelium–extracellular matrix interaction Cell surface receptor receptor Homeostasis, development and inflammation	Lung function (FEV_1/FVC) Emphysema	[24, 48]
SFTPD	10q22.3	rs721917 rs2245121 rs911887 rs6413520 rs7078012	Host defence Surfactant metabolism A promising COPD biomarker	Lung function (FEV_1) Emphysema	[55]
ADAM19	5q33.3	rs1422795	Immune defense Inflammatory process Extracellular matrix breakdown and reconstruction	Lung function (FEV_1/FVC) Airflow obstruction	[48]
RARB	3p24.2	rs1529672	Active form of vitamin A Embryonic morphogenesis Cell growth and differentiation Tumor suppression	Lung function (FEV_1/FVC)	[56]

FAM13A: family with sequence similarity 13 member A; TGFB2: transforming growth factor beta 2; HHIP: hedgehog interacting protein; HTR4: hydroxytryptamine receptor 4; CHRNA3/5: cholinergic receptor nicotinic alpha 3/5; MMP12: matrix metalloprotein 12; AGER: advanced glycosylation end-product specific receptor; SFTPD: surfactant protein D; ADAM19: a disintegrin and metalloprotease 19; RARB: retinoic acid receptor beta; FEV1: forced expiratory volume in 1 second; FVC: predicted forced vital capacity.

is not merely caused by smoking.[7] The heterogeneity of COPD indicates that individuals have different susceptibilities to it, and biological networks consisting of genes and proteins are important determinants of COPD.

Severe alpha-1 antitrypsin deficiency was the first documented genetic determinant of COPD.[22] Over the past decade, a large number of studies have focused on the role of genetic variants in COPD using classical genome-wide association studies (GWAS) that have successfully identified many genomic regions in association with COPD susceptibility, as listed in Table 2.[23, 24] Studies of candidate genes have linked specific loci to phenotypes of COPD;[25,26] therefore, the newly discovered susceptibility genes could provide novel insights into COPD pathogenesis. However, identifying functional variants and key genes within these regions remains a major challenge. Therefore, an integrated approach combining GWAS and other omics sequencing data (transcriptomics, epigenetic analysis, proteomics, metabolomics, etc.) may be required to provide a more comprehensive view of the genetic architecture of COPD.[27]

DRUG TARGETS FOR PERSONALIZED MEDICINE

COPD, characterized by non-type 2 inflammation, is usually corticosteroid insensitive. Smoking cessation attenuates the accelerated decline in lung function in patients with COPD. Long-acting β2-adrenergic receptor agonists and long-acting muscarinic acetylcholine are beneficial for alleviating symptoms.[11] However, although drugs produce effective bronchodilation, no drugs are available to considerably reduce disease progression or mortality. This has prompted a concerted search for new treatments and biomarkers that can monitor the responses of specific patients. Many potential drug targets have been implicated in the development of COPD animal models. These targets include inflammation medium inhibitors, antioxidants, and kinase inhibitors, in particular, drugs targeting cell regeneration, microbial colonization, and corticosteroid resistance.[28,29] However, some targets have so far not shown clear clinical benefits in COPD, albeit they are limited by side effects owing to the widespread distribution of intracellular targets.[57]

In summary, animal models should be developed to accurately simulate the distinct clinical features of COPD. It is necessary to optimize the existing modeling program from an array of aspects, such as the choice of combined factors, species and susceptibility genes, construction of multi-locus gene-editing animals, and application of advanced imaging tools. Based on analysis of phenotypes, mechanisms of the disease should be further investigated in combination with multi-omics analyses. With a better understanding of the disease, more potent targets may be discovered in the future, which can reduce the burden of comorbidities and treatment costs. The development of biomarkers could optimize the selection of patient populations for clinical trials, and therapies can be tailored to an individual patient's disease profile for personalized medicine.

Source of Funding

This work was supported by grants from the National Key Research and Development Program of China 2022YFF0710800 and 2018YFC1313600, Major International (Regional) Joint Research Project of China 81820108001, National Natural Science Foundation of China 81670029, Jiangsu Key Principal Investigator of Medicine ZDRCA2016018, and Jiangsu Provincial Project 333 for Cultivation of High-Level Talents (Leading Talents of the Young and Middle-Aged) BRA2019078 (to L. Zhou).

Conflict of Interest

The authors have nothing to disclose.

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How to cite this article: Jiang J, Xu S, Chen Z, Liu W, Zhang L, Li J, *et al*. Animal Models: An essential tool to dissect the heterogeneity of chronic obstructive pulmonary disease. J Transl Intern Med 2023; 11: 4-10.