Original Research Article

ENVIRONMENTAL EPIDEMIOLOGY

OPEN

Assessing the impact of exposome on the course of chronic obstructive pulmonary disease and cystc fibrosis

The REMEDIA European Project Approach

Manon Benjdir[a], Étienne Audureau[a,b], Ariel Beresniak[c], Patrice Coll[d], Ralph Epaud[a,e,f], Kristina Fiedler[g], Bénédicte Jacquemin[h], Laurent Niddam[i], Spyros N. Pandis[j,k], Gerhard Pohlmann[l], Torkjel M. Sandanger[m], Kai Simmons[n,o], Mette Sørensen[p,q], Patrick Wagner[r], and Sophie Lanone[a*]

Abstract: Because of the direct interaction of lungs with the environment, respiratory diseases are among the leading causes of environment-related deaths in the world. Chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF) are two highly debilitating diseases that are of particular interest in the context of environmental studies; they both are characterized by a similar progressive loss of lung function with small bronchi alterations, and a high phenotypic variability of unknown origin, which prevents a good therapeutic efficacy. In the last years, there has been an evolution in the apprehension of the study of diseases going from a restricted "one exposure, one disease" approach to a broader concept with other associating factors, the exposome. The overall objective of the REMEDIA project is to extend the understanding of the contribution of the exposome to COPD and CF diseases. To achieve our aim, we will (1) exploit data from existing cohorts and population registries to create a unified global database gathering phenotype and exposome information; (2) develop a flexible individual sensor device combining environmental and biomarker toolkits; (3) use a versatile atmospheric simulation chamber to simulate the health effects of complex exposomes; (4) use machine learning supervised analyses and causal inference models to identify relevant risk factors; and (5) develop econometric and cost-effectiveness models to assess the costs, performance, and cost-effectiveness of a selection of prevention strategies. The results will be used to develop guidelines to better predict disease risks and constitute the elements of the REMEDIA toolbox. The multidisciplinary approach carried out by the REMEDIA European project should represent a major breakthrough in reducing the morbidity and mortality associated with COPD and CF diseases.

Key Words: Exposome; Chronic ostructive pulmonary disease; Cystic fibrosis

Introduction

In Europe, the burden of lung disease remains as high today as it was at the turn of the millennium and will probably remain so for several decades.[1] The associated economic cost for respiratory diseases in the 28 EU countries amounts to more than 380 billion euros per year, including direct primary and hospital care.[2] Lungs interact directly with the environment, and respiratory diseases are among the leading causes of environment-related deaths in the world: 1.4 million deaths per year.[3] Air quality is of paramount importance in this context, and the impact of air pollution on human health is a global concern. According to the State of Global Air report,[4] more than 90% of the World's population is exposed to $PM_{2.5}$ (particulate matter with a mean aerodynamic diameter below 2.5 μm) concentrations exceeding the guideline limit of 10 μg/m^3 set by the World Health Organization (WHO). Air pollution is now considered as one of the major causes of environment-related deaths, accounting for 7 million each year.

Among noncommunicable respiratory diseases, chronic obstructive pulmonary disease (*) and cystic fibrosis (CF) are

[a]*University Paris-Est Créteil, INSERM, IMRB, Creteil, France;* [b]*Public Health Department, Clinical Research Unit (URC), Hôpital Henri-Mondor, Assistance Publique Hôpitaux de Paris (APHP), Créteil, France;* [c]*Data Mining International SA, Geneva, Switzerland;* [d]*Laboratoire Interuniversitaire des Systèmes Atmosphériques, UMR CNRS 7583, Université de Paris et Université Paris-Est Créteil, Institut Pierre Simon Laplace, Créteil, France;* [e]*Department of general pediatrics, Centre Hospitalier Intercommunal de Créteil, Créteil, France;* [f]*Center for Rare Lung Diseases (RESPIRARE), Créteil, France;* [g]*INSERM Transfert, Paris, France;* [h]*Université Rennes 1, INSERM, EHESP, Irset (Institut de Recherche en Santé, Environnement et Travail)—UMR_S 1085, Rennes, France;* [i]*Wellspring Kft, Budapest, Hungary;* [j]*Institute of Chemical Engineering Sciences, FORTH, Patras, Greece;* [k]*Department of Chemical Engineering, University of Patras, Patras, Greece;* [l]*Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany;* [m]*Department of Community Medicine, Health Faculty, UiT-the Arctic University of Norway, Tromsø, Norway;* [n]*Lipotype GmbH, Dresden, Germany;* [o]*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany;* [p]*Diet, Genes and Environment, Danish Cancer Society Research Center, Copenhagen, Denmark;* [q]*Department of Natural Science and Environment, Roskilde University, Roskilde, Denmark; and* [r]*KU Leuven, Laboratory for Soft Matter and Biophysics, Leuven, Belgium.*

The authors declare that they have no conflicts of interest with regard to the content of this report.

Data and code can be obtained from the corresponding author.

The REMEDIA project has received funding from the European Union's Horizon 2020 Research and Innovation Program under grant agreement No 874753.

What this study adds?

By basing its methodology on the concept of exposome, the REMEDIA project will be one of the first to study a complex set of environmental parameters on the evolution of two lung diseases of opposite origin but sharing many features: Chronic Obstructive Pulmonary Disease (environmental origin) and cystic fibrosis (genetic origin). To this end, the REMEDIA partners will analyze data from five cohorts, develop a sensor, and simulate exposomes in preclinical models. Thanks to its multidisciplinary approach, REMEDIA's ultimate achievement will produce a plan with strategic recommendations for policy makers and regulators to reduce the risk of lung diseases.

two highly debilitating diseases that are of particular interest in the context of environmental studies, given their "opposite" roots in the current knowledge; environmental for COPD and genetic for CF (Figure 1). Indeed, COPD is currently the third leading cause of death in the world, accounting for 5.3% of all deaths,[5] with no curative treatment available to date. In 2015, 3.2 million people died of COPD worldwide, which represents an increase of approximately 12% compared with 1990. COPD is characterized by persistent and usually progressive airflow limitation resulting from a combination of a diffuse disease of small airways and the destruction of the pulmonary parenchyma leading to emphysema. COPD patients present multiple variations of their phenotype that may imply different origins and underlying pathophysiological mechanisms, which complicates medical treatment. The main risk factor for developing COPD is smoking; about 40%–50% of lifetime smokers will develop COPD. However, at equivalent tobacco consumption, not all smokers develop clinically significant COPD, suggesting that other risk factors should be taken into account, such as occupational exposures, air pollution (indoor/outdoor), as well as genetic factors that may modify the individual risk of developing COPD.[6] CF is the most common autosomal recessive inherited genetic disorder in the White population (1 in 2,500 births). Although probably under-reported in developing countries, CF affects at least 70,000 persons worldwide, including about 36,000 individuals in the EU. CF places a heavy burden on people in terms of morbidity, health care utilization, and mortality. Although advances in basic science and new treatments such as CFTR protein repair therapy have decreased mortality, CF remains an incurable disease for which the average life expectancy does not exceed 50 years. CF causes lung, pancreatic, digestive and hepatic disorders expressed at different levels of severity leading to high phenotype variability. Obstructive pulmonary disease in CF patients accounts for about 80% of their mortality. CF is associated with a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene, leading to dysfunction of the CFTR protein. To date, more than 2,000 mutations in the *CFTR* gene have been identified.[7] Importantly, the genotypic variability associated with CF does not fully explain the phenotypic variability. Indeed, although CF is a monogenic disease, there is a wide clinical diversity in patients; the evidence suggests that some manifestations of the disease are related to the severity of the underlying CFTR mutations as well as environmental and genetic modifiers, which currently remain of an overall unknown nature.

COPD and CF are therefore two chronic noncommunicable diseases that are characterized by a similar progressive loss of lung function with small bronchi alterations, as well as by a high phenotypic variability of unknown origin, which prevents a good therapeutic efficacy for these two diseases.[8,9] In the last years, there has been an evolution in the apprehension of the study of diseases going from a restricted "one exposure, one disease" approach to a broader concept with other associating factors, thus corresponding to the concept of exposome ("*life-course environmental exposures, including lifestyle factors, from prenatal period onwards*").[10] This shift in approach supports research of broader sets of parameters to capture highly complex, variable, and dynamic exposures in both space and time. The REMEDIA project participates in the common EU effort to broaden knowledge about the human exposome in the course of COPD and CF disease. Deciphering the impact of the exposome throughout life on the phenotypic variability of COPD and CF could represent a major breakthrough in reducing the morbidity and mortality associated with these two noncurable diseases through identification of modifiable risk factors on which preventive action could be implemented.

Project description

Aim

The overarching goal of the REMEDIA project is to extend the understanding of the influence of the exposome on the course of COPD and CF (Figure 2), and thus identify predictive markers of the development, exacerbation, and expression of these diseases. The specific questions that will be answered by the REMEDIA project are the following:

- Are there specific exposomes associated with particular COPD and CF phenotypes (severity, morbidity, exacerbations, comorbidities)?
- Are there specific exposomes linked to lung function trajectories and maximal achieved lung function at early adulthood (taken as an early determinant of COPD and CF outcome)?
- How do similar exposomes impact COPD and CF?

Who is in the study?

Bringing together 13 partners from nine European Union countries, the REMEDIA consortium is composed of public and academic research organizations, hospitals, and small and medium-sized enterprises. The consortium has a clear multidisciplinary dimension gathering several fields of expertise with the collaboration of epidemiologists and public health specialists (DCS, UiT, INSERM, KA), adult and pediatrician lung specialists (CHIC, INSERM), physico-chemist of the atmosphere (LISA, FORTH), biologists (INSERM, Lipotype, CEGX), engineer for sensor development (FhG, KUL), economists (DMI), ethics and legal specialists (WLSG), and management and technology transfer (IT). REMEDIA Consortium has therefore a clear multidisciplinary dimension that is essential for the successful completion (Figure 3).

What has and will be measured?

The methodological approach of REMEDIA is based on an inclusive three-step research plan. The project will first start with data integration activities. Following the advances and progress of this first phase, the project will continue with activities related to experimental work. Finally, the last phase of the project will be devoted to the activities related to analytical and computational work. Data will be collected continuously throughout the project and will feed into subsequent phases (Figure 4).

Data integration

This part of the project is dedicated to gathering and, whenever legally and ethically possible, integrating within a unified database, all preexisting health and environmental data relevant to REMEDIA's research objectives from the participating cohorts and registries, in line with local and European regulatory requirements and in accordance with each study data sharing

*Corresponding Author. Address: INSERM U955/Institut Mondor de Recherche Biomédicale, Faculté de Santé, 8 rue du Général Sarrail, 94010 Créteil, France. E-mail: sophie.lanone@inserm.fr (S. Lanone).

Environmental Epidemiology (2021) 5:e165

Received: 7 September 2020; Accepted 28 June 2021

Published online 6 August 2021

DOI: 10.1097/EE9.0000000000000165

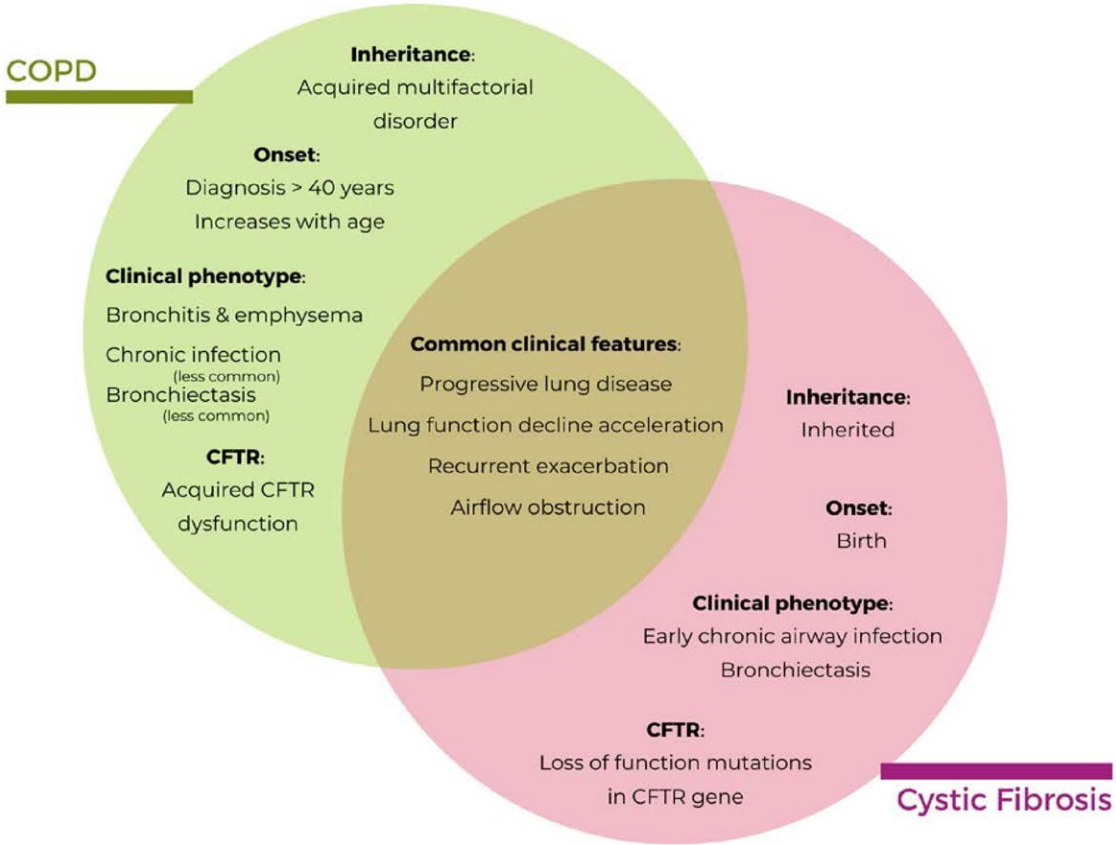

Figure 1. Differences and similarities between COPD and CF adapted from De Rose et al.[8]

policy. Data integration will be achieved from five well-designed existing cohorts and population registries (Table 1); 2 mother-child cohorts (*Avon Longitudinal Study of Parents and Children*—ALSPAC and *Perturbateurs Endocriniens: Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance*—PELAGIE), the French CF registry (covering all lifelong ages), and 2 adult cohorts (*Danish Cancer Health*—DCH and *Norwegian Women and Cancer Cohort*—NOWAC).[11]

The database will include preexisting data on exposome and lung/disease features from the participating cohorts/registries and will be further enriched with public environmental and contextual information to be matched with subjects geocoded residence and newly conducted OMICs analyses in targeted case-control studies. Collected data will aim at covering a broad representation of the exposome, including whenever possible specific and general external exposome components, for example, exposure to atmospheric pollutants (particulate matter, nitric oxides, ozone, volatile organic compounds, and carbon monoxide), life style (diet, physical activity, sleep duration, smoking, alcohol & drugs consumption, pets in the home), occupational exposure, socio-economic level (individual social economic status, house crowding, living conditions, median household income), UV, surrounding natural space, built environment, surrounding traffic, as well as internal exposome components (e.g., data from microbiome, lipidomic and epigenetic analyses). Clinical information regarding COPD and CF endpoints (age at onset/diagnosis, lung function, comorbidities, exacerbations, mortality) will also be collected. Regarding diagnosis of COPD, it is noteworthy that it is not a rare disease, with an estimated worldwide mean prevalence of 13.1% (CI 95% 10.2–15.6%) and 12.4% (8.8–16.0%) in Europe.[12] Thus, thanks to the general population data available in both Denmark and Norway cohorts for which we have accurate exposome data, we will be able to identify COPD patients through linkage to high-quality national hospital patient registries. In addition for the Danish cohort, we will be able to use the national COPD register to refine/confirm COPD diagnosis (>2000 COPD patients expected). Regarding biologic material, all cohorts will have blood samples available for omics analyses. In the NOWAC cohort as an example, blood samples were

Figure 2. Graphical depiction of the overall aim of REMEDIA.

Figure 3. REMEDIA partner map.

collected during 2003–2006 and citrate plasma is available for lipidomics and buffy coat for DNA-Methylation analysis. Blood samples 0–4 years before COPD diagnosis will be included in this project (200 case–control pairs).

This data integration stage will be accompanied by a first round of statistical analyses to describe subjects' main characteristics and data availability on a per cohort basis. This is particularly important as exposome/disease/lung function information will not be available similarly in all cohorts. Lung function for instance (e.g., FEV_1) will be available in some cohorts at annual- (CF registry), three (ALSPAC), or one (PELAGIE) time point(s), although not directly available in NOWAC and DCH cohorts. Likewise, granularity levels for geocoded information will vary, ranging from neighborhood-level (ALPSAC) to broader geographic units (e.g., French department for CF registry). Given such heterogeneity between data sources in availability and granularity of information, analyses will be primarily performed on per cohort basis with no cross-cohort studies; efforts will be made to use findings yielded from one cohort to inform analyses led in other ones (e.g., validating findings from ALSPAC using aligned/simplified information from PELAGIE). Depending on the magnitude of missing data rates and under the assumption of data missing at random, missing data imputation using nonparametric missForest or multiple imputation by chained

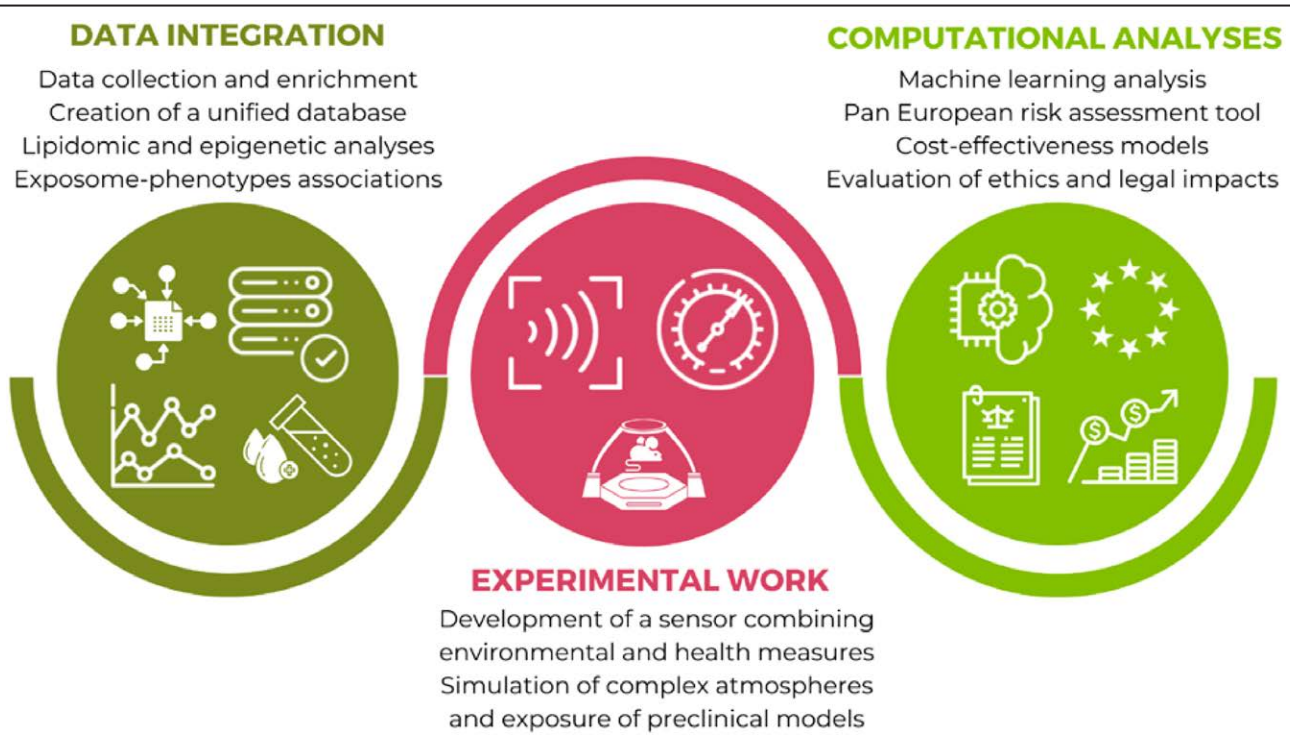

Figure 4. REMEDIA global methodological approach.

Table 1.

Participating cohorts and registries

Main topic/disease of interest	Cohort	Country	Number of subjects
Lung function in the youth	ALSPAC cohort	United Kingdom	14,500 families in the Bristol area, United Kingdom
Lung function in the youth	PELAGIE cohort	France	3,400 pregnant mothers included in 2002 and followed-up with children
CF	French Registry of CF patients	France	6,750+ children and adults with CF
COPD	DCH cohort	Denmark	57,053 individuals between 50–65 years at enrollment in 1993–1997
COPD	NOWACcohort	Norway	170,000 women aged 30–70 years at recruitment from 1991

equations methods will be considered. Analyses at this stage will also aim at documenting cooccurrence/correlations between the different exposome components and to perform unsupervised clustering approaches to characterize typical COPD and CF phenotypes. As a first step before conducting global analyses (see below), we will then explore exposome-phenotype associations through an exposome-wide association study (ExWAS) approach to identify and prioritize candidate exposome components showing promising correlations with COPD and CF severity/course and lung function evolution (Figure 5).

Experimental work

Following the initial progress of the data integration phase, experimental work will be conducted, with two main objectives:

- The development of two flexible, integrated sensors; an environmental sensor together with a biosensor. Based on an extended literature review on relevant markers, a first prototype of environmental sensor device will be developed and tested under laboratory conditions. In parallel, a prototype of biosensor device will be developed, and further tested in healthy volunteers and adapted to molecular biomarkers relevant to COPD and CF patients. Finally, these two devices will operate in parallel and will be validated within COPD and CF volunteers. The idea is that both sensors-the environmental sensor and the biosensor—will eventually be placed at home or generally in the "life environment" of CF and COPD patients. We expect that the environmental parameters and the concentration of inflammation show a "positive" correlation. A proof of concept for the biosensor will be carried out in healthy volunteers, by measuring the previously agreed molecules. The performance of the biomarker toolbox will be validated against common assays (gold standard) for these molecules as a reference.
- The quantification of the health impacts of the exposomes identified in the other parts of REMEDIA in naïve COPD or CF preclinical models. For COPD, we will use the classical model of daily exposure to cigarette smoke for up to 6 months, using a Teague machine.[13] For CF, we will use two different mouse strains: Scnn1-Tg and ΔCftr^{tm1Eur} mice. These models are largely described in CF literature.[14] More specifically, our strategy is to couple an atmospheric simulation chamber where the complexity of real atmosphere will be reproduced, to a cabinet where preclinical models will be exposed. Two complementary simulation chambers will be used in the project: a mobile Teflon chamber (ASC, @FORTH) that can interact directly with the solar radiation, and a stainless steel chamber of higher volume (CESAM, @LISA), which allows longer-term exposures that are essential for the simulation of chronic lung diseases. Other components of the exposome (diet, physical activity, noise, etc.) will also be implemented, depending on findings obtained in the *data integration* part of the project. Finally, preclinical models will be exposed to the different exposomes at various periods of their lives and health endpoints such as lung function (trajectories) will be studied (Figure 6).

Global analyses and risk assessment

Building upon database construction, data integration, and experimental work, the objectives here are to conduct global analyses on the relationships between exposome risk components and subsequent lung diseases occurrence or severity (risk assessment), and to develop new predictive tools and etiologic models (causal inference modeling) useful for assessing the main risk factors and implementing effective preventive interventions. To that end, analyses will rely on both conventional regression and advanced machine learning approaches to fully capture patient's complexity by determining the best predictors in isolation and combination and establishing their predictive value on the outcomes of interest. Causal relationships and potential pathways between exposome and individual features will be explored using multilevel mediation models by structural equation modeling or based on the counterfactual framework. Moreover, an economic assessment of the relation between exposome and the course of COPD and CF diseases will be performed, to evaluate the cost-effectiveness of candidate preventive strategies, keeping in mind ethics and legal impact of such strategies.

Findings to date

Bio- and environmental sensors

To assess the inflammatory status of COPD and CF patients, the project partners have identified two molecular biomarkers that are present in exhaled air. For both types of molecules, we are developing selective bioreceptors on the basis of aptamers and molecularly imprinted polymers. These receptors can be synthesized with protocols suitable for batch production, they offer regeneration capacity, and they can be integrated with electronic detection principles. For measurements on exhaled breath in its gas phase, microbalances are the detection principle of choice although we opt for impedance spectroscopy in case of condensed, liquid exhalate. With the measurement on condensed, liquid exhalate, we will determine the concentration of the molecular biomarkers hexanal and nitrotyrosine.[15,16] Adding a third biomarker to this panel is currently under consideration. Briefly, the receptors for both markers are deposited onto an array of planar, miniature-scale gold electrodes on glass chips. Binding of the marker molecules from the supernatant liquid to the receptor layer results in a concentration-dependent increase of the impedance signal. The calibration curves, that is, the relationship between concentration and signal, will be established by spiking the liquid exhalate of healthy volunteers with appropriate doses of both marker molecules. The accuracy of the biosensor-derived concentration data on exhalate of CF- and COPD patients will furthermore be validated by mass-spectrometry based reference analysis.

For exposome measurement in the environment, it was decided to focus on seven relevant parameters: temperature, relative humidity, particulate matter, nitric oxides, ozone, volatile organic carbon, and carbon monoxide. The respective measurement ranges and accuracies were derived from literature. In the final device, all sensors will be integrated in a miniaturized module. There exists already two commercially available

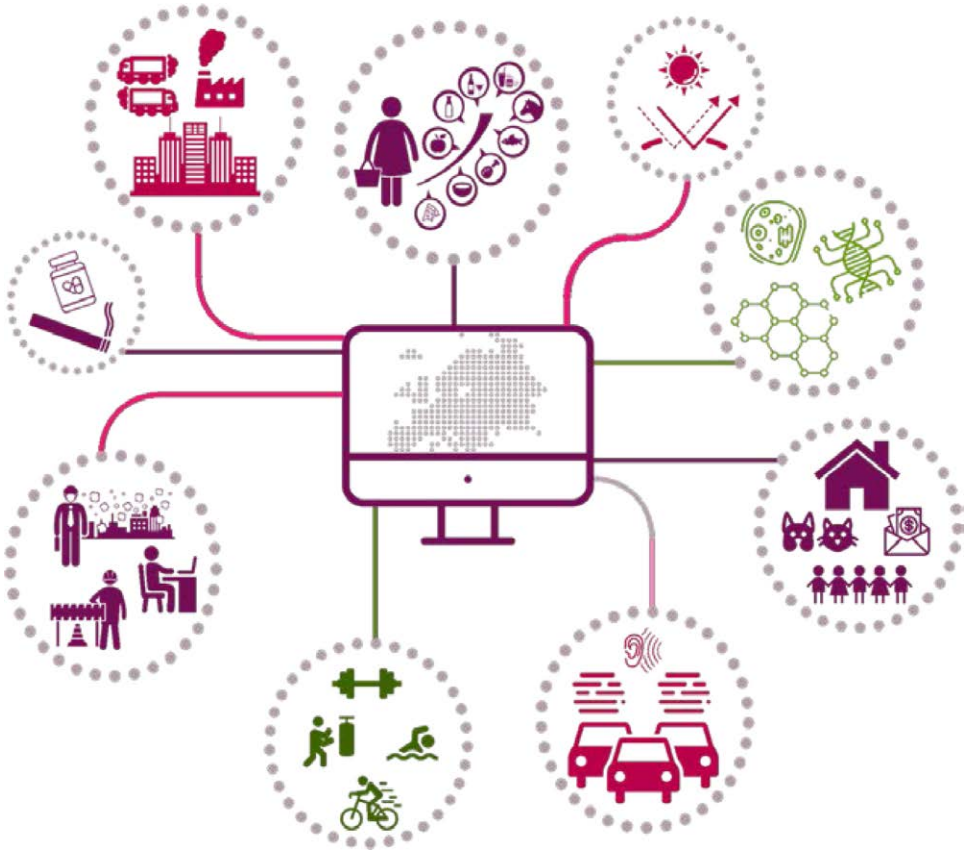

Figure 5. Data collected and exposome components studied during REMEDIA.

platforms and one proprietary platform from Fraunhofer IZM, which cover the sensor functionalities partially. The platforms will be compared—especially with respect to modularity. Then, the most suitable platform will be used to setup a system with a complete set of modular hardware components for environmental monitoring. A first nonminiaturized prototype of the environmental sensor will be available at the beginning of 2021.

Data integration

Although not planned in the initial design of REMEDIA, we implemented a new study to the project in the context of the recent Covid-19 pandemic. Indeed, as nonpharmacological interventions (NPI), including lockdowns, have been used to address the COVID-19 pandemic, the exposome profile of individuals may be affected in the months following such measures owing to alterations in diet, physical activity, sedentary behavior, and hand hygiene habits. In this context, we aim to assess the impact of the first lockdown in France on the cohort of CF patients studied in REMEDIA, in terms of supply and utilization of care for patients with CF (cancelation or postponement of consultations, hospitalizations, or treatment such as antibiotic courses). Secondary objectives include the assessment of compliance, anxiety, and stress (at risk of being affected by COVID-19

Figure 6. Experimental set-up.

or at risk of being treated less well), patient's quality of life, including the disease symptoms, living conditions, knowledge of the disease and the measures taken, and evaluate the knowledge, experience, and social representations of the risk of Covid-19.

Assessment will be performed using a quantitative analysis with a national online questionnaire submitted to the 4,000 French CF patients >14 years old and a qualitative analysis with interviews of 15 representative patients, conducted by recorded video-conference during and after the lockdown.

In 1 month, thanks to the French CF association "Vaincre la mucoviscidose" and all the French CF centers (CRCM), 1,200 patients were connected, resulting in 747 complete and usable questionnaires.

Simulation of complex atmospheres

To support the experimental work relative to the quantification of the health impacts of the exposomes identified in patients, we have implemented an innovative approach, with the aim of realistically simulating, in the laboratory, the atmospheric mixture in all its complexity, thus keeping the ability to control, reproduce, and carefully characterize the experimental conditions. As an illustration, to study the myriad of products arising from the atmospheric oxidation of primary organic compounds, we developed the following protocol, using the CESAM chamber (4.2 m^3 stainless steel atmospheric simulation, evacuable down to a few 10^{-7} atm, temperature-controlled between +15°C and +60°C): the experimental protocol consists in the continuous injection in the CESAM simulation chamber operated as a slow flow reactor, of relevant mixtures of primary pollutants (mainly nitrogen oxides, organic compounds from a representative mix of anthropogenic emissions, sulphur dioxide, soot, inorganic salts, and potentially mineral dust particles if needed, e.g., to simulate Beijing's atmosphere at low concentrations [ppb levels] in the air). The residence time of simulated air parcels in the experimental volume is fixed to 4 hours, to represent air masses of regional scale. During this time, the synthetic mixture is exposed to an artificial solar irradiation, allowing secondary pollutants such as ozone, nitric acid, formaldehyde, peroxyacetyl nitrate (PANs) as well as complex polyfunctional organics including Secondary Organic Aerosol (SOA) to be produced and to reach their chemical steady state. Living organisms are exposed to constant flows of such a mixture during time scales of a week to address their health effect. To scale the pollutant concentrations in the initial mix, we were able to run a predictive model to estimate the concentrations of all gaseous and particulate species in CESAM, after a residence time of 4 hours in the chamber. The model takes into account the injection of 9 precursors (gaseous chemicals) in the simulation chamber: nitrous acid, toluene, acetylene, ethane, benzene, meta-xylene, ortho-xylene, para-xylene, ethyl-benzene, and n-pentane. After 20 hours of flowing and mixing in the chamber (this duration can be reduced to a few hours by increasing the concentrations and flows of precursors), the targeted concentrations of pollutants are obtained. Then, the light simulating the sunlight irradiation is switched on and taking into account about 1,500 reactions involving around 3,000 chemicals, the model predicts what will be the photostationary concentrations in the chamber for any of those photoproducts. Once the requested steady-state CESAM experimental conditions are reached, a desired volumetric flowrate is transferred to the compartment where the living organisms are exposed, thanks to an overpressure of 11 millibars inside CESAM chamber. The path between the chamber and the exposure devices is minimized to preserve the numerous reactive chemicals present in the simulated atmosphere. The CESAM chamber is equipped with a comprehensive set of analytical instruments and benefits from the instrumental environment dedicated to atmospheric chemistry provided by CNRS-LISA,[17] especially gaseous pollutants analyzers (O_3, NO, NO_2, SO_2, CO, CO_2, COV…) and aerosols properties (mass and

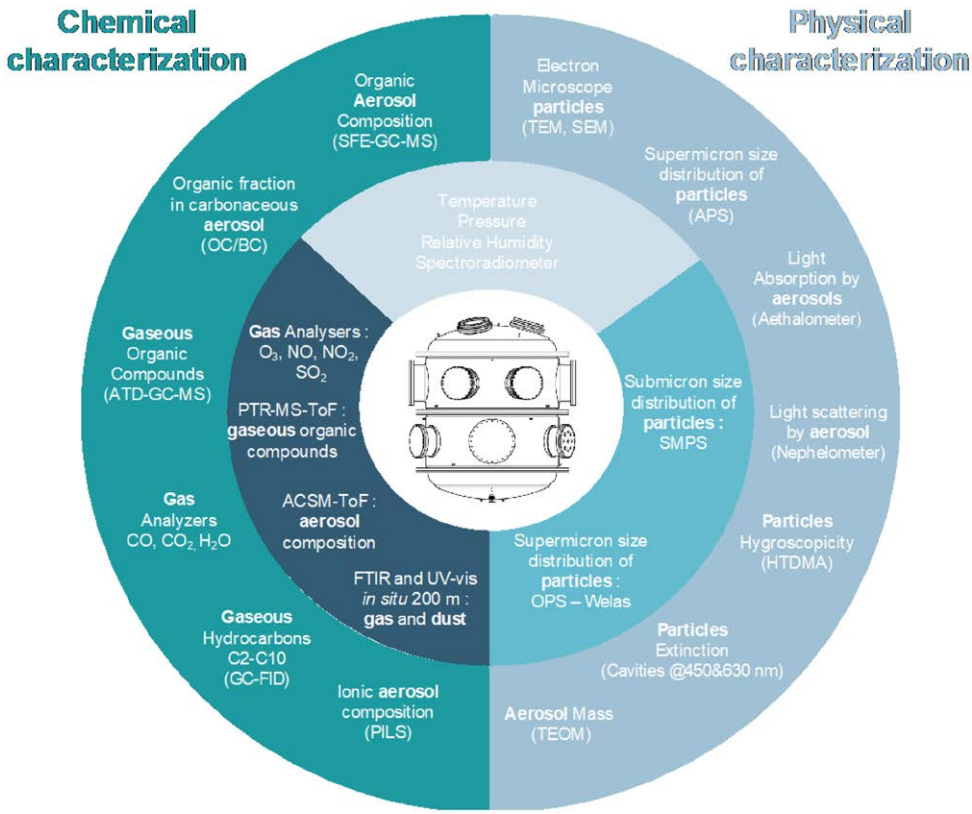

Figure 7. CESAM analytical environment.

size distributions, hygroscopicity…). This allows us to accurately and precisely measure the time evolution of the pollutant concentrations within the different exposure compartments (Figure 7).

There are three different ways that can be used to introduce solid particles in the CESAM chamber:

(a) Inorganic aerosols: inorganic particles are continuously injected in CESAM from a salt(s) solution (e.g., $[NH_4]_2SO_4$ solution in the $10^{-4}/10^{-3}$ Mol.l^{-1}) using a commercial atomizer (TSI 3076 atomizer), with a transfer rate to CESAM relevant to the targeted final concentration.

(b) Soot introduction: for the control of continuous soot introduction in CESAM, a second chamber directly connected to CESAM is used as a soot reservoir; the "CESAM Auxiliary Chamber" or CAC. CAC has a volume of 2 m^3 and is equipped with a pumping system, a fan and a 0–1000 Torr pressure sensor. During the introduction of soot into the CAC, the two chambers are isolated from each other. The use of a miniCAST (model 5201A) allows us to fill the CAC with a known mass of soot. This quantity is determined according to the final amount of soot desired in CESAM. When the introduction is complete, the two chambers are connected to each other and a fixed nitrogen flow allows the introduction of soot continuously from the CAC to CESAM. The frequency of introduction of soot by the CAST is determined according to the dilution rate in CESAM.

(c) Dust particles: about 15 g of soil sample (e.g., Gobi desert soil in the case of Beijing's simulation) is placed in a Büchner flask and shaken for about 30 minutes at 100 Hz by means of a sieve shaker (Retsch AS200). The dust suspension in the flask is then injected into the chamber by flushing it with N_2 at 10 L min^{-1} for about 10–15 minutes, whilst continuously shaking the soil.

Strength and limitations—main challenges

REMEDIA has several strengths (Figure 8). The first strength is to study COPD and CF diseases in parallel, in the context of the exposome. The ground-breaking concept here relies on the simultaneous consideration of two noncommunicable diseases of opposite roots with regards to the current understanding of the exposome contribution. Moreover, we evaluate exposures at different age-windows which addresses the issue of sensitive exposure windows as well. Many individual exposures have already been studied in the context of lung diseases. It may be particularly true for COPD, but not as much for CF, as the latter is mainly considered to be the only result of genetic alteration(s). However, what REMEDIA will bring is the notion of comprehensive analysis of external exposome as a whole, meaning individual components together with their interactions. Moreover, we choose to study not only (classical) epigenetic modifications, but also lipidomic data, which is a real asset. Finally, the econometric model developed in the project will complete the exposure assessment by linking environmental risk factors with health outcomes. Overall, we strongly believe that the results obtained in the framework of REMEDIA will provide guidance for decision makers involved in public health and environmental policies.

Another strength of REMEDIA is its Consortium, that brings a clear multidisciplinary dimension, with complementary expertise from different academic and industrial backgrounds.

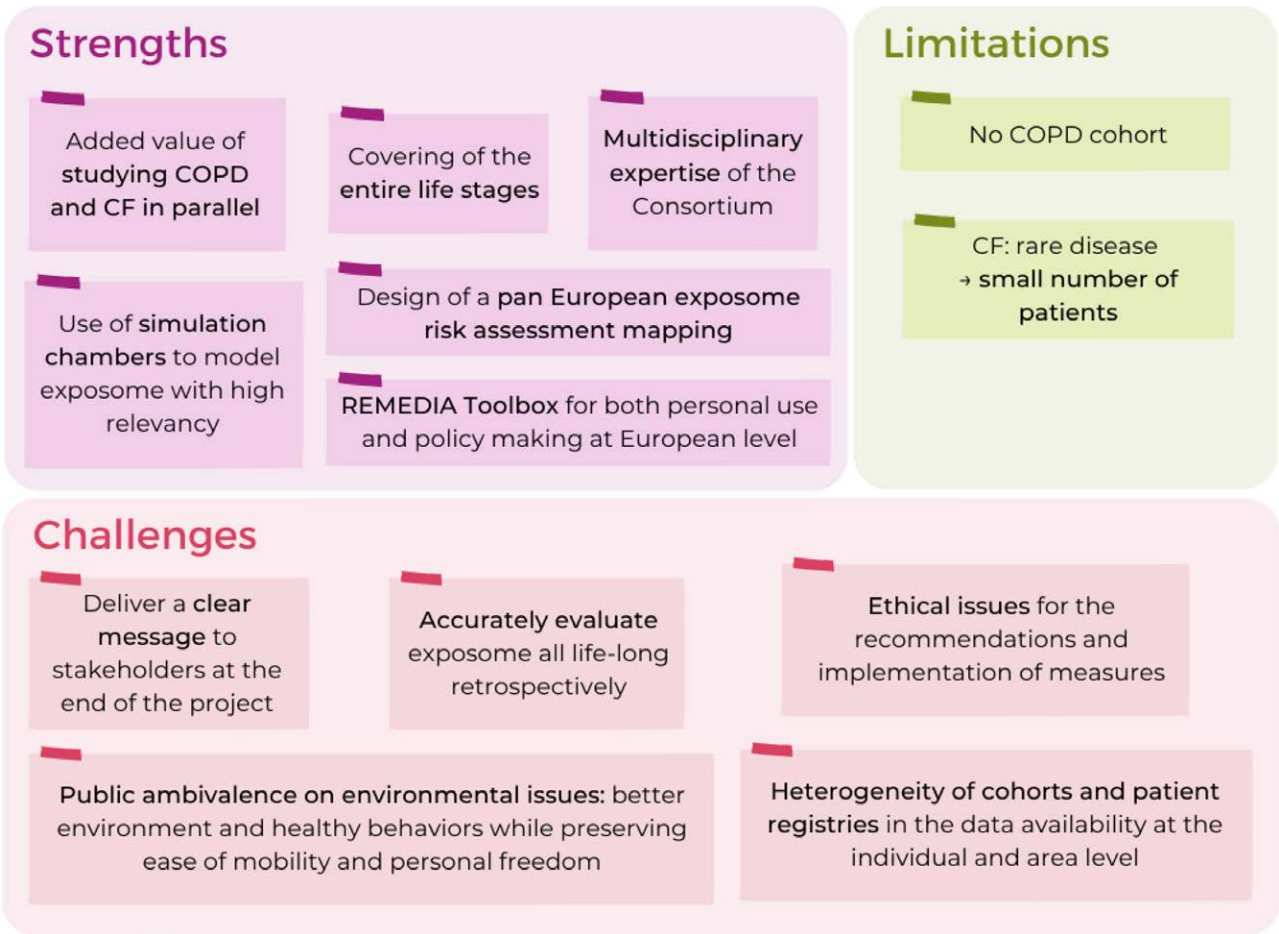

Figure 8. Strengths, limitations and challenges raised in the REMEDIA project.

Together, REMEDIA's partners cover the value chain necessary to achieve our objectives. Although the Consortium as a whole is new, several partners have already worked together, which brings a strong cohesion already in the first steps of REMEDIA. Moreover, throughout the consortium, the expertise of one partner will complement that of the other. For example, LISA and FORTH have a complementary expoertise on simulation chambers that will be used in REMEDIA. In addition, it is expected that the results for the two classes of biomarkers (epigenetics and lipidomics) will be compared between partners to arrive at the "best" configuration in terms of biomarkers to be tested in the workpackage dedicated to sensor development. The use of the two atmospheric simulation chambers is also an asset; it allows the exposure of the models to realistic and well-controlled atmospheres with multiple primary and secondary gas and aerosol pollutants for extended periods of time. The two chambers, one made out of steel and the second made out of transparent Teflon can together cover a wide range of the atmospheric chemical space.

By exploiting existing cohorts and population registries, the REMEDIA project will deliver a toolbox integrating the different items developed throughout the project (Figure 9). The toolbox will thus provide key elements to design effective tailored prevention actions and care programs. The toolbox will include developments for personal use (sensor and risk assessment models embeddable into applications and IoT devices) and for policy making at European level (risk assessment mapping and continuous data collection). Ethical and legal reviews as well as econometric analyses to model and simulate the impact of future prevention programs will also be considered, as they are essential to support policy changes and justify immediate to yield future benefits.

As an exposome study, the REMEDIA project will face a set of challenges. One of these challenges is to accurately measure the totality of exposures to which an individual is subjected throughout life. Indeed, the exposome is constantly evolving and depends on numerous parameters: nature and characteristics of pollutants, combinations of exposures, individual behaviors, occupational health, and socio-economic context at least. These temporal and spatial variabilities combined with individual variability (body response to exposures) make the effects of several exposures still difficult to assess. In the framework of the REMEDIA project, this might result in the difficulty of identifying the health effects of exposome. The lack of homogeneity in the different cohorts and population registries is a second challenge to address. Indeed, the data availability at the individual level (e.g., health-related behaviors) and at the area level (e.g., atmospheric pollution indicators) may vary from country to country.

Finally, environmental issues now occupy a prominent place in the public debate. Numerous individual, associative and governmental initiatives arise in favor of a better environment and healthy behaviors. However, there is still public ambivalence about how to achieve these ambitions although preserving ease of mobility and personal freedom. Moreover, these interventions, acting on the broad spectrum of potential risk factors involved, require action in a number of interconnected and often conflicting regulatory areas (infrastructure, employment, industry, transport, education). Research in the field of environmental health and the production of results and recommendations for decision-makers should therefore consider this ambivalence by providing assessments of the proposed interventions (ethical and legal review) and the costs involved (cost-effectiveness assessment). Dissemination strategy will specifically target Policy-Makers at national and European levels (Health Ministries, European Commission, European, Parliament) and Regulatory Agencies, as well as international organizations (WHO) to design effective preventive actions.

Conclusion

Following the previous millennium great discoveries in the field of the human genome, the human exposome appears to be more and more crucial in understanding the development and exacerbation of many pathologies. The multidisciplinary approach carried out by the REMEDIA European project will allow to assess the impact of exposome components throughout life on the phenotypic variability of COPD and CF, which will

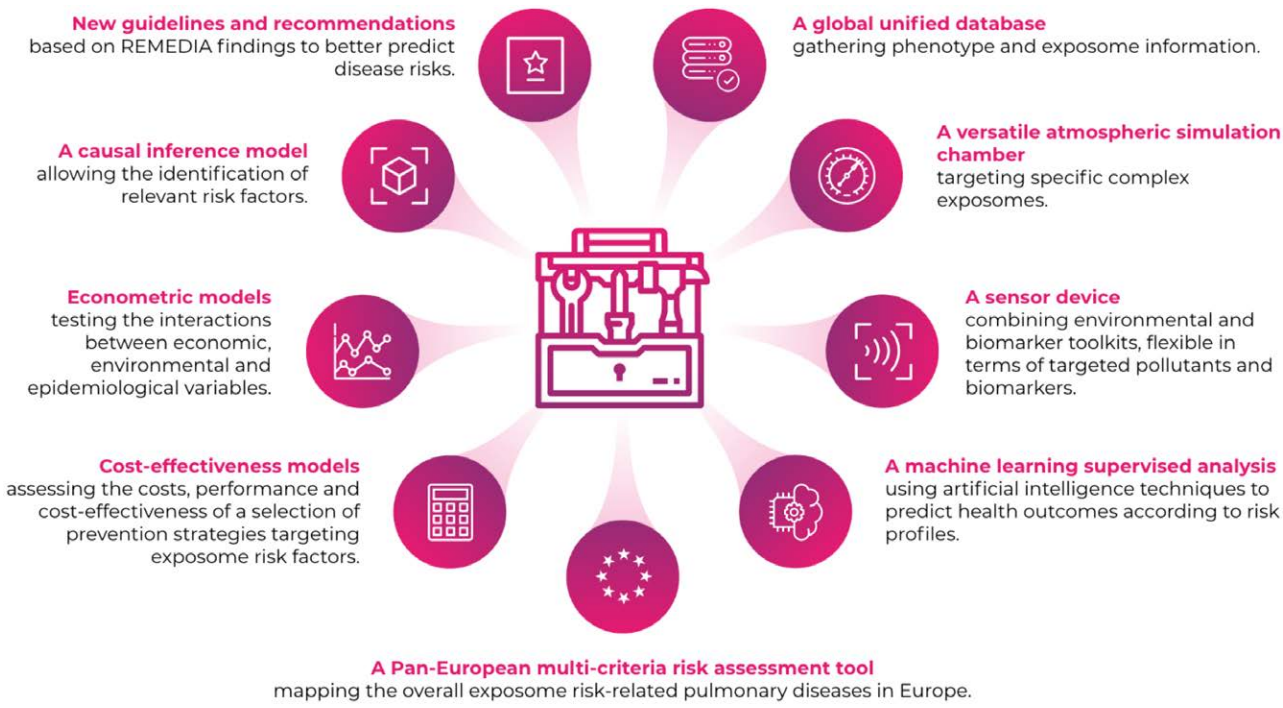

Figure 9. Schematic of the REMEDIA toolbox.

represent a major breakthrough in reducing the morbidity and mortality associated with these two untreatable diseases, and will lead to the identification of specific risk factors for implementing preventive public health programs.

References

1. European Respiratory Society. *The Burden of Lung Disease. European Lung White Book*. Available at: https://www.erswhitebook.org/chapters/the-burden-of-lung-disease/. Accessed 15 July 2020.
2. European Respiratory Society. *The Economic Burden of Lung Disease. European Lung White Book*. Available at: https://www.erswhitebook.org/chapters/the-economic-burden-of-lung-disease/. Accessed 16 July 2020.
3. Prüss-Üstün A, Wolf J, Corvalán C, Bos R, Neira M. *Preventing Disease through Healthy Environments: A Global Assessment of the Burden of Disease from Environmental Risks*. 2nd ed. World Health Organization; 2016.
4. Climate & Clean Air Coalition. *State of Global Air 2019: a Special Report on Global Exposure to Air Pollution and Its Disease Burden*. Available at: https://www.stateofglobalair.org/sites/default/files/soga_2019_report.pdf. Accessed 31 August 2020.
5. GOLD. *2020 Global Strategy for Prevention, Diagnosis and Management of COPD*. Available at: https://goldcopd.org/wp-content/uploads/2019/12/GOLD-2020-FINAL-ver1.2-03Dec19_WMV.pdf. Accessed 20 May 2020.
6. Melén E, Guerra S. Recent advances in understanding lung function development. *F1000Res*. 2017;6:726.
7. Fanen P, Wohlhuter-Haddad A, Hinzpeter A. Genetics of cystic fibrosis: CFTR mutation classifications toward genotype-based CF therapies. *Int J Biochem Cell Biol*. 2014;52:94–102.
8. De Rose V, Molloy K, Gohy S, Pilette C, Greene CM. Airway epithelium dysfunction in cystic fibrosis and COPD. *Mediators Inflamm*. 2018;2018:1309746.
9. Fernandez Fernandez E, De Santi C, De Rose V, Greene CM. CFTR dysfunction in cystic fibrosis and chronic obstructive pulmonary disease. *Expert Rev Respir Med*. 2018;12:483–492.
10. Wild CP. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev*. 2005;14:1847–1850.
11. Lund E, Dumeaux V, Braaten T, et al. Cohort profile: The Norwegian Women and Cancer Study–NOWAC–Kvinner og kreft. *Int J Epidemiol*. 2008;37:36–41.
12. Blanco I, Diego I, Bueno P, Casas-Maldonado F, Miravitlles M. Geographic distribution of COPD prevalence in the world displayed by Geographic Information System maps. *Eur Respir J*. 2019;54:1900610.
13. John G, Kohse K, Orasche J, et al. The composition of cigarette smoke determines inflammatory cell recruitment to the lung in COPD mouse models. *Clin Sci (Lond)*. 2014;126:207–221.
14. Wilke M, Buijs-Offerman RM, Aarbiou J, et al. Mouse models of cystic fibrosis: phenotypic analysis and research applications. *J Cyst Fibros*. 2011;10 (Suppl 2):S152–S171.
15. Corradi M, Rubinstein I, Andreoli R, et al. Aldehydes in exhaled breath condensate of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2003;167:1380–1386.
16. Balint B, Kharitonov SA, Hanazawa T, et al. Increased nitrotyrosine in exhaled breath condensate in cystic fibrosis. *Eur Respir J*. 2001;17:1201–1207.
17. The CESAM chamber home page. *Chamber for Experimental Multiphase Atmospheric Simulation*. Available at: https://www.cesam.cnrs.fr. Accessed 31 August 2020.