

Of flies, mice and men: a systematic approach to understanding the early life origins of chronic lung disease

Susanne Krauss-Etschmann,^{1,2} Andrew Bush,³ Saverio Bellusci,⁴ Guy G Brusselle,⁵ Sven Erik K Dahlén,⁶ Stefan Dehmel,¹ Oliver Eickelberg,¹ Greg Gibson,⁷ Machteld N Hylkema,⁸ Petra Knaus,⁹ Melanie Königshoff,¹ Clare M Lloyd,¹⁰ Paolo Macciarini,¹¹ Arnaud Mailleux,¹² Benjamin J Marsland,¹³ Dirkje S Postma,¹⁴ Graham Roberts,^{15,16} Christos Samakovlis,¹⁷ Janet Stocks,¹⁸ Joke Vandesompele,¹⁹ Matthias Wjst,¹ John Holloway¹⁶

For numbered affiliations see end of article

Correspondence to

Dr Susanne Krauss-Etschmann, Comprehensive Pneumology Center, Helmholtz Center Munich and Children's Hospital of Ludwig-Maximilians University, Max-Lebsche Platz 31, Munich 81377, Germany; susanne.krauss-etschmann@helmholtz-muenchen.de

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ABSTRACT

Despite intensive research efforts, the aetiology of the majority of chronic lung diseases (CLD) in both, children and adults, remains elusive. Current therapeutic options are limited, providing only symptomatic relief, rather than treating the underlying condition, or preventing its development in the first place. Thus, there is a strong and unmet clinical need for the development of both, novel effective therapies and preventative strategies for CLD. Many studies suggest that modifications of prenatal and/or early postnatal lung development will have important implications for future lung function and risk of CLD throughout life. This view represents a fundamental change of current pathophysiological concepts and treatment paradigms, and holds the potential to develop novel preventative and/or therapeutic strategies. However, for the successful development of such approaches, key questions, such as a clear understanding of underlying mechanisms of impaired lung development, the identification and validation of relevant preclinical models to facilitate translational research, and the development of concepts for correction of aberrant development, all need to be solved. Accordingly, a European Science Foundation Exploratory Workshop was held where clinical, translational and basic research scientists from different disciplines met to discuss potential mechanisms of developmental origins of CLD, and to identify major knowledge gaps in order to delineate a roadmap for future integrative research.

DEVELOPMENTAL ORIGINS OF RESPIRATORY HEALTH AND DISEASE: THE CONCEPT

Broad interest in developmental processes, and their relationship to later human health, emerged in 1986 when Barker *et al* demonstrated a positive geographical correlation of infant mortality with adult death rates from coronary heart disease in England and Wales.¹ The 'Barker hypothesis' proposed that low birth weight, as a proxy for intra-uterine nutrient restriction, might be the major determinant for cardiometabolic disease in adulthood.² This concept was further fuelled by retrospective observations from the 'Dutch Hunger Winter' in 1944, where severe undernourishment during pregnancy was associated with increased

susceptibility for obesity, arterial hypertension or type II diabetes in the offspring.³ Over time, it became clear that not only a highly deficient environment, but also other intrauterine exposures, such as environmental toxicants and maternal disease states are associated with later disease risks. Accordingly, the concept of 'Developmental Origins of Health and Disease' suggests that the fetus makes physiological adaptations in response to the intrauterine environment to prepare itself for postnatal conditions. These adaptations may be beneficial if intra- and extra-uterine environment correspond to each other, but will be disadvantageous if the postnatal environment has changed in the meantime, or has been wrongly 'predicted'. While these hypotheses have been mainly elaborated in relation to cardiometabolic diseases and behaviour, epidemiological studies suggest that the risk of developing chronic lung disease (CLD) is equally modified through perinatal exposures. This has been most convincingly shown for asthma⁴⁻⁶ and COPD,⁷⁻⁹ where a number of factors that are either protective, or predispose to the disease, have been identified. It is suspected that these modifications are, at least in part, mediated by epigenetic mechanisms.¹⁰ This is important, as epigenetic modifications have been shown to be transmitted trans-generationally,¹¹ suggesting that 'memory' of previous environmental exposure in a population may result in increased disease risk in subsequent generations. Moreover, the importance of epigenetic processes also provides an opportunity for intervention, as animal models have shown, that interventions such as maternal dietary modification in pregnancy have the potential to modify epigenetic programming and reduce subsequent disease risk.¹² Given all of this, we would like to propose a Developmental Origins of Respiratory Health and Disease (DORHaD) concept to focus activity on an area with the potential to deliver improved respiratory health outcomes.

However, the extent to which disease risks are established during embryo-fetal life and the underlying mechanisms are far from understood, and susceptible developmental windows have not been adequately explored. If interventions that will improve the health of the population with respect

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to CLD are to be developed, it is essential that these knowledge gaps are addressed. To this end, a multidisciplinary team involving experts from developmental biology, immunology, cell signalling, lung biology, stem cell biology, (epi)genetics, epidemiology and clinical science, met in an ESF-Exploratory Workshop (<http://www.esf.org/activities/exploratory-workshops/medical-sciences-emrc.html?year=2011&domain=EMRC>) to develop a conceptual framework for future research on developmental origins of CLD. This review aims to demonstrate why and how interactions among clinical and basic research disciplines can generate a fruitful cross-fertilisation of ideas to treat and/or prevent CLD.

LUNG DEVELOPMENT—AND WHAT HAPPENS IF IT GOES WRONG

Development and molecular pathways

Mammalian lung development begins by formation of a bud from the floor of the primitive foregut around the fourth week of human gestation and continues through the pseudoglandular, canalicular, sacular and alveolar stages far into postnatal life.¹³ During this highly controlled process, the endoderm gives rise to the different epithelial cell lineages, while structural components of the lung, such as pulmonary vessels or airway smooth muscle, originate from the mesoderm. Once fully mature, the lung will harmonise the functions of around 40 different cell types.¹⁴ The formation of such a complex organ requires the precise spatiotemporal orchestration of multiple signalling molecules during development. Animal models of airway development demonstrate that early disruption of critical signalling pathways can lead to abnormal pulmonary phenotypes after birth. The first example for a phylogenetically conserved genetic program orchestrating airway branching derives from the *Drosophila* branchless (*Bnl*)¹⁵ and its mammalian homologue, Fibroblast Growth Factor 10 (*FGF10*). Both, *Bnl* and *FGF10*, are expressed and secreted by the surrounding mesenchyme of the epithelial buds and control their outgrowth during early lung development through their epithelial receptors, *Breathless* and *FGFR2-IIIb*, respectively. The crucial contribution of *FGF10* signalling to branching morphogenesis is demonstrated by *Fgf10*^{-/-} and *Fgfr2b*^{-/-} embryos which have lung agenesis.¹⁶

An entirely different level of regulation of lung development involves microRNAs (miRNAs) that suppress gene expression at the posttranscriptional level through binding of target mRNAs, and regulate essential cellular functions, such as growth, differentiation and programmed cell death. Hence, miRNAs have already been shown to control the development of broncho-alveolar tissues,¹⁷ and also respond to environmental factors, such as LPS¹⁸ or tobacco smoke,¹⁹ while others participate in epigenetic regulation.²⁰ Nonetheless, it is largely unknown how microRNAs precisely regulate lung development or contribute to airway tissue remodelling.

From developmental pathways to disease

Through experimental disruption of developmental pathways, we now know that molecular pathways involved in lung development can be recapitulated later in life, provoking pathologic changes in complex respiratory diseases. For example, canonical Wnt/ β -catenin signalling has recently been identified as a key pathway for early lung development.²¹ Wnt ligands and their receptors are expressed in a highly cell-specific manner in the developing lung and recede during later stages. In experimental and human fibrotic lung disease, the Wnt/ β -catenin signalling pathway is aberrantly reactivated, and has been linked *in vitro*

and *in vivo* with alveolar epithelial cell repair mechanisms via one of its target genes Wnt1-inducible signalling pathway protein 1 (*WISP1*).²² *In vivo* depletion of *WISP1* results in significant attenuation of lung fibrosis and improved survival in preclinical studies. Wnt-signalling genes have been further associated with impaired lung function in two childhood asthma cohorts,²³ and activation of this pathway led to enhanced proliferation of bronchial epithelial cells.²⁴ However, the precise contribution of each of the 19 Wnt ligands, 10 Frizzled receptors and signalling components to development and disease remains to be elucidated.

Studies in animal models and patients with pulmonary fibrosis or COPD have also provided evidence for a reactivation of other developmental pathways, such as TGF- β , or PTEN/PI3kinase/Akt-mediated signalling.²⁵

From growth to lung function

For normal lifelong lung function, the prerequisites are: (1) normal lung function at birth; (2) normal growth in lung function until the adult plateau at 20–25 years of age; and (3) no accelerated deterioration from the plateau. Cohort studies have established that lung function either tracks or deteriorates, but never improves, after the preschool years.^{26–28} Thus, lung function in adult life is critically dependent on in utero and postnatal lung development.

An important antenatal factor affecting newborn lung function is maternal smoking, which causes structural effects on the developing lung.²⁹ A study in more than 13 000 people showed that childhood disadvantage defined by either maternal or paternal asthma, childhood asthma, maternal smoking and childhood respiratory infections predicts worse adult lung function, a faster rate of lung function decline, and a greater prevalence of COPD.³⁰ Other important influences include maternal antibiotic³¹ and paracetamol³² use, maternal psychological stress,³³ nutrition,³⁴ diabetes, hypertension³⁵ and exposure to pollution.³⁶

Equally important is to comprehend whether patterns of early somatic growth are associated with altered respiratory and immune development. Anthropometric measurements at birth, and markers of fetal growth, have been linked epidemiologically to asthma.^{37–38} In a recent large cohort study, longitudinal prenatal and infant growth patterns were related to wheeze and atopy at age 3 years in 1548 children.³⁹ A rapid growth trajectory during 11–19 weeks of gestation followed by late gestation growth faltering was associated with atopy, suggesting that influences affecting fetal growth may also alter immune development. In contrast, a lower early fetal growth trajectory was associated with non-atopic wheeze, possibly reflecting an association with smaller airways.

With continuously improving survival rates, preterm birth is a further and increasingly important cause of early onset airflow obstruction. Recent evidence suggests that even non-ventilated late preterm infants from 33 weeks of gestation onwards show impaired lung function at least until the age of 8–9 years.⁴⁰

In children, it is essential to distinguish between the effects of disease from those of growth and development. However, structural assessments of lung and airway dimensions do not necessarily reflect functional changes in lung growth and development, or vice versa. There are many tests of lung function, each interrogating a different airway region. For example, spirometry gives information about proximal airway disease, whereas, tests of gas mixing, such as lung clearance index (LCI) are more sensitive to distal airway problems. It is also not

possible to distinguish alveolar number from alveolar size, as the cause of lung volume changes and measured lung volume changes may be artefactual due to airway disease. Thus, DLCO may give a measure of alveolar surface area, but the measurement is also vulnerable to impaired gas mixing. Determining whether there is disease in the silent regions of the lung (distal airways) or at the silent ages (2–6 years) is also a challenge. It is now possible to record flow volume curves in infancy (raised volume rapid thoracic compression technique), in pre-schoolers (using incentive spirometry) and from school age right through into old age (conventional spirometry). LCI can be performed at all ages, and after the first year of life, has the same normal range independent of age or height. This technique has been shown to be most sensitive in cross-sectional and longitudinal studies in cystic fibrosis,⁴¹ and preschool LCI is strongly predictive of school-age lung function abnormalities.⁴²

In summary, tools for measuring lung function from birth to old age are available. However, while there is a good understanding of spirometry, it is relatively insensitive, in particular, to early airway disease. On the other hand, LCI is more sensitive, but we lack experience in interpreting changes. Nonetheless, suitable tools to monitor lung growth from birth onward are available and will help to identify clinical subphenotypes to ensure investigation of relevant outcomes, both, in humans and in preclinical studies.

TOOLS OF THE TRADE—WHERE ARE WE NOW?

As highlighted above, an increased understanding of the early life influences on lung development is needed to devise new strategies aimed at the primary prevention of lung disease. This requires development of cellular and animal models to investigate biological mechanisms and test effectiveness of preventative strategies.

Transgenerational studies in rodents have mainly focused on global maternal under/overnutrition, restriction of selective nutrients, psychological stress, or endocrine disorders, looking at cardiovascular and behavioural outcomes in some instances until the F2 generation.⁴³ Evidence for transgenerational transmission of risk for lung disease emerged when offspring from tobacco smoke-exposed dams showed airway remodelling and decreased pulmonary expression of Wnt-signalling molecules and targets.⁴⁴ A recent key study has demonstrated that nutritive supplementation of dams with methyl donors affects asthma risk across two subsequent generations, and involves differential methylation of several pulmonary genes including *runx-related transcription factor 3* (*Runx3*) which, if not silenced, controls airway inflammation.¹⁰ Similarly, exposure of dams to diesel exhaust particles during gestation heritably alters innate immune responsiveness in F1 and F2 generations.⁴⁵

As well as for prenatal exposures, appropriate postnatal challenge models are required. For example, the role of commensal bacteria in guiding the normal development of immune homeostasis has received attention,⁴⁶ and neonatal allergen exposure models highlight how airway remodelling, inflammation and airway hyper-reactivity developed in parallel, rather than sequentially,⁴⁷ supporting previous observations in humans.⁴⁸ These observations underscore the importance of developing mouse models that are not only relevant to clinical disease phenotypes, but also address different developmental phases.

Despite the comparatively quick generation time of murine models and ample availability of molecular tools, refining of susceptible developmental windows for single types of exposures,

and assessing risk transmission until F3/F4 generations is time consuming and expensive in mice.

The formation of the respiratory organ of the fruit fly, *Drosophila melanogaster*, demonstrates the basic principles of branch patterning and tube growth, and allows the analysis of airway maturation at the genome-wide level with or without perturbations and simplified interventions. Additionally, the transparency of fly embryos allows the analysis of cellular activities of airway epithelial cells in unprecedented detail by live imaging.

A comparative genomics approach among *D melanogaster* and higher organisms can generate novel testable molecular pathways. These can then be studied in detail in higher organisms and during defined developmental stages. Such a preselection strategy would also justify efforts to move to large animal models where the anatomy and physiology of the lung, the placenta, pregnancy characteristics and dietary requirements have closer resemblance to humans. Additionally, 'high throughput' identification of relevant types of exposures might be feasible in lower organisms, such as *D melanogaster*.

Animal models need corresponding cell culture systems, which, although lacking the context of physiology, allow the biology of single molecules and pathways to be studied at high resolution. Available molecular tools are applicable also for two, triple or 3D culture systems to study the effects of intercellular crosstalk on cell-specific changes in gene or protein expressions in the context of exposures. These may be further complemented by whole-organ cultures, such as isolated ventilated perfused lungs or bronchial ring studies, to study a specific function in intact organs independent of the remainder organism, while maintaining the local physiological homeostasis. However, performing such analyses across species, and in combination with *in vitro* and *in vivo* disease models requires a considerable collaborative effort among researchers.

WHY WE SHOULD TALK—A ROADMAP FOR FUTURE RESEARCH

In understanding the mechanisms underlying DORHaD, a major challenge will be the development of effective bridging systems to establish collaboration among basic and clinical sciences in order to combine expertise in human conditions with detailed knowledge of complementary model systems. In addition, computational biology is required to handle and analyse complex datasets in a systems biology approach.

The discovery and functional description of pathways during lung morphogenesis by developmental biologists have already led to the generation of hypotheses that are testable in translational studies, for example, knowledge of the role of developmental Wnt pathways in the pathobiology of pulmonary fibrosis has led to the identification of WISP1 as a potential therapeutic target.

Vice versa, reverse translation of clinical and epidemiological findings is mandatory to drive novel basic research. Thus, the identification of a number of maternal environmental exposures during pregnancy, as risk factors for adverse lung development, have formed the basis of mechanistic studies.⁴⁹

These examples demonstrate that, while human clinical, genetic and environmental epidemiological studies can identify potential causes of disease, these will require the use of animal models to provide supporting evidence and to investigate biological mechanisms. Proving causality from early environmental exposures on development and disease will require a systematic, focused and concerted effort. A roadmap for future

A roadmap for joint research on early origins of chronic lung disease

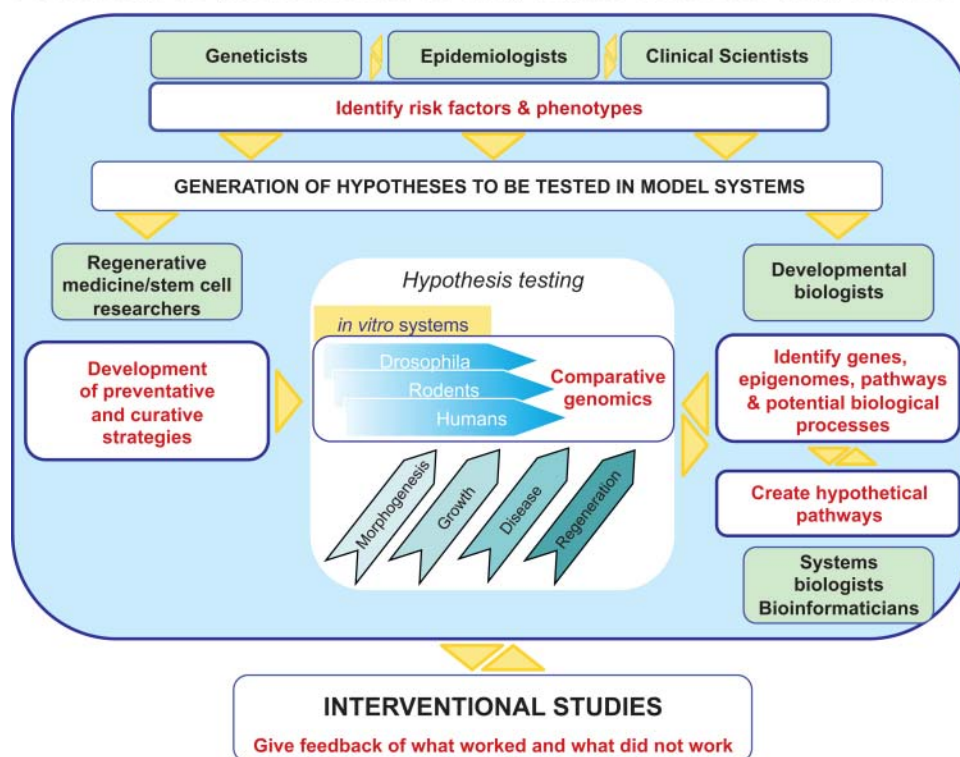


Figure 1 From observational studies, clinicians and epidemiologists create hypotheses to be tested in model systems. Developmental biologists discover molecular pathways and susceptible developmental windows relevant for later disease. This can lead to the identification of candidate treatment targets and testable hypotheses. A comparative genomics approach across model systems will allow to create hypothetical pathways and biological processes. Their role in development can again be tested in model systems. Researchers in regenerative medicine, such as stem cell biologists, work on practical applications that can be applied in interventional studies in the future.

Box 1 Questions that can only be answered in a concerted effort

- ▶ What is the normal ontology of relevant cell populations, and is their activity perturbed prior to the onset of disease?
- ▶ How do critical cell types and molecular events that initiate CLD respond to exposures?
- ▶ Which exposures affect lung development, how do they do so, and what are the crucial developmental time points?
- ▶ What are the underlying mechanisms of transgenerational transmission of disease risks, including molecular pathways, cellular interactions and epigenetic changes?
- ▶ What are relevant animal models that permit to track the risk for specific chronic pulmonary diseases across generations?
- ▶ What are triggers of normal development and of successful repair processes to regenerate lung tissue in the chronically injured lung?
- ▶ What is the effect of gender on developmental processes and subsequent risk of disease, and the underlying mechanisms?
- ▶ Which early life events affect the initial rate of increase and later rate of decline in lung function?

cross-disciplinary research is depicted in figure 1. This, or similar approaches, should lead to answers to the most pressing questions (box 1) and will, hopefully, allow us to move on from

experimental and observational studies to interventional cohort studies to prevent or cure CLD and deliver the promise of the DORHaD approach.

Author affiliations

- ¹Comprehensive Pneumology Center, Helmholtz Center Munich, Munich, Germany
- ²Children's Hospital of the Ludwig Maximilians University, Munich, Germany
- ³Imperial College and Royal Brompton Hospital, London, UK
- ⁴Excellence Cluster in Cardio-Pulmonary Systems, Department of Internal Medicine II, University of Giessen Lung Center, Giessen, Germany
- ⁵Department of Respiratory Medicine, Laboratory for Translational Research in Obstructive Pulmonary Diseases, Gent University Hospital, Belgium
- ⁶Institute of Environmental Medicine, Division of Physiology, The Unit of Experimental Asthma and Allergy Research, Karolinska Institutet, Sweden
- ⁷Center for Integrative Genomics, Georgia Institute of Technology, School of Biology, Atlanta, Georgia, USA
- ⁸Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
- ⁹Institute of Chemistry and Biochemistry, Freie Universitaet, Berlin, Germany
- ¹⁰Leukocyte Biology, National Heart & Lung Institute, Imperial College London, London, UK
- ¹¹Department of General Thoracic and Regenerative Surgery and Intrathoracic Biotransplantation University Hospital Careggi, Florence, Italy
- ¹²INSERM, U700, Université Paris Diderot, Sorbonne Paris Cité, Paris, France
- ¹³Faculty of Biology and Medicine, University of Lausanne, Service de Pneumologie, Lausanne, Switzerland
- ¹⁴Department of Pulmonology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
- ¹⁵NIHR Respiratory Biomedical Research Unit, University Hospital Southampton NHS Foundation Trust, Southampton, UK
- ¹⁶Human Development & Health and Clinical & Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK
- ¹⁷Developmental Biology, The Wenner-Gren Institute, Stockholm University, Sweden
- ¹⁸Portex Unit: Respiratory Physiology and Medicine, UCL Institute of Child Health, London, UK

¹⁹Center for Medical Genetics, Gent University Hospital, Gent, Belgium and Biogazelle, Zwijnaarde, Belgium

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