# Epigenetics and pulmonary diseases in the horizon of precision medicine: a review

Giuditta Benincasa[1], Dawn L. DeMeo[2], Kimberly Glass[2], Edwin K. Silverman[2] and Claudio Napoli[1,3]

**Affiliations**: [1]Dept of Advanced Medical and Surgical Sciences (DAMSS), University of Campania "Luigi Vanvitelli", Naples, Italy. [2]Channing Division of Network Medicine and the Division of Pulmonary and Critical Care Medicine, Dept of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. [3]Clinical Dept of Internal and Specialty Medicine (DAI), University Hospital (AOU), University of Campania "Luigi Vanvitelli", Naples, Italy.

**Correspondence**: Claudio Napoli, University Dept of Advanced Medical and Surgical Sciences (DAMSS), Clinical Dept of Internal Medicine and Specialistic Units, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy. E-mail: claudio.napoli@unicampania.it

@ERSpublications
**Network medicine approaches, supporting the integrative analysis of global epigenetic changes, clinical features and environmental factors, provide new hypotheses for pathogenesis of chronic lung diseases and lead to new interventions for prevention** https://bit.ly/3kHND9m

**Cite this article as:** Benincasa G, DeMeo DL, Glass K, *et al*. Epigenetics and pulmonary diseases in the horizon of precision medicine: a review. *Eur Respir J* 2021; 57: 2003406 [https://doi.org/10.1183/13993003.03406-2020].

ABSTRACT Epigenetic mechanisms represent potential molecular routes which could bridge the gap between genetic background and environmental risk factors contributing to the pathogenesis of pulmonary diseases. In patients with COPD, asthma and pulmonary arterial hypertension (PAH), there is emerging evidence of aberrant epigenetic marks, mainly including DNA methylation and histone modifications which directly mediate reversible modifications to the DNA without affecting the genomic sequence. Post-translational events and microRNAs can be also regulated epigenetically and potentially participate in disease pathogenesis. Thus, novel pathogenic mechanisms and putative biomarkers may be detectable in peripheral blood, sputum, nasal and buccal swabs or lung tissue. Besides, DNA methylation plays an important role during the early phases of fetal development and may be impacted by environmental exposures, ultimately influencing an individual's susceptibility to COPD, asthma and PAH later in life. With the advances in omics platforms and the application of computational biology tools, modelling the epigenetic variability in a network framework, rather than as single molecular defects, provides insights into the possible molecular pathways underlying the pathogenesis of COPD, asthma and PAH. Epigenetic modifications may have clinical applications as noninvasive biomarkers of pulmonary diseases. Moreover, combining molecular assays with network analysis of epigenomic data may aid in clarifying the multistage transition from a "pre-disease" to "disease" state, with the goal of improving primary prevention of lung diseases and its subsequent clinical management.

We describe epigenetic mechanisms known to be associated with pulmonary diseases and discuss how network analysis could improve our understanding of lung diseases.

This article has supplementary material available from erj.ersjournals.com

Received: 6 Sept 2020 | Accepted: 10 Nov 2020

## Introduction

The molecular changes underlying the pathogenesis of many chronic pulmonary diseases are not fully understood, and potentially relevant targets for risk stratification and prevention are not well-established. In recent years, genome-wide association studies (GWAS) have identified many genomic regions that contain genetic variants significantly associated with complex, nonmalignant pulmonary diseases, including COPD, asthma and pulmonary arterial hypertension (PAH) [1–3]. However, although the development of polygenic risk scores has improved disease risk prediction, much of disease susceptibility remains unexplained by the identified variants [1–3]. Indeed, specific epigenetic marks, especially DNA methylation, histone modifications and micro-RNAs (miRNAs), can also affect the endophenotypes underlying the onset of COPD, asthma and PAH [4–7]. Furthermore, epigenetic-sensitive changes are acquired with ageing and impacted by environmental exposures or inherited by mitotic and meiotic cell division (transgenerational effects), providing a longstanding "memory" of the early life exposures experienced during fetal development that may ultimately impact an individual's susceptibility to cardiopulmonary diseases later in life [5, 8–12]. Most studies on the variability of epigenetic marks have focused on specific sites or regions of the genome. However, considering epigenetic marks in a network framework may be more informative than site-specific analyses [13–16]. A network is structured in "nodes" and "edges" which derive from applying advanced bioinformatic algorithms to omics data. This strategy has the potential to decipher the relationship between clinical data and the genes, proteins and metabolites that may play a critical role in disease pathogenesis. Remarkably, epigenetic changes modulate the interface between genomic and environmental risk factors and may drive an "accelerated" ageing of lungs [17]. Thus, investigating epigenetic changes by using network analysis in longitudinal cohorts may identify novel, noninvasive biomarkers that are useful for precision medicine applications in pulmonary disease [18–20]. Network medicine approaches combine advanced omics platforms, potent bioinformatic algorithms and clinical data [13–16]. Many network medicine applications have used the molecular interactome as a key tool to discover how the interplay between genes and the environment may differentially perturb protein–protein interactions (PPIs) [13–16]. In addition, correlation-based and gene regulatory networks have provided valuable insights into disease pathogenesis [13–16]. In this article, we summarise and discuss current evidence for the role of epigenetics in COPD, asthma and PAH, and how insights from epigenetic data are already pointing to potential biomarkers useful for prevention and personalised therapy. We also motivate the need to develop and apply network approaches to better understand the role of epigenetics and epigenetic variability in pulmonary diseases.

## A focus on molecular basis of epigenetic mechanisms

Epigenetic-sensitive modifications, including DNA and RNA methylation, histone modifications and noncoding RNAs can modulate gene expression without changing DNA sequence in a spatiotemporal manner [10, 21] (figure 1). Remarkably, exposures during early development or post-natal life may perturb the individual "epi-interactome", defined mainly by DNA methylation changes, leading to higher risk of developing complex diseases [8, 9, 22–27].

DNA methylation is a chemical reaction mediated by DNA methyltransferase (DNMT) enzymes belonging to three protein families including DNMT1, 2 or 3, which catalyse the addition of methyl groups at the 5′ carbon of cytosines into CpG dinucleotides [28]. Usually, there is an inverse relationship between promoter DNA methylation and gene expression [29, 30]

To balance methylation levels, the demethylases belonging to the 10–11 translocation (TET) family of DNA dioxygenases (TET1/2/3) remove the methyl groups from cytosine bases favouring an open chromatin state associated with active gene expression [31].

Acetylation and methylation changes are the main histone modifications which are harboured at specific amino acid positions (mainly lysine) of the N-terminal region, where they form a "code" deciphered by other chromatin remodelling complexes [32]. The global acetylation level is regulated by two classes of enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs) which specifically increase and decrease the number of acetyl groups into histone tails, respectively. As downstream effects, HATs can favour increased gene expression, whereas HDACs are associated with gene silencing [32]. Otherwise, histone methylation is catalysed by the family of lysine methyltransferase enzymes [33] Generally, the effect of histone methylation on gene expression varies according to the specific amino acid position; for example, lysine 4 methylation is associated with gene activation [34] whereas lysine 9 methylation is associated with gene silencing [35].

miRNAs are small RNA molecules (21–22 nucleotides) acting at the post-transcriptional level where they can bind to the 3′ untranslated regions of targeted mRNAs to avoid their translation in protein products [36]. miRNA-mediated regulation is very complex because generally a single miRNA regulates hundreds of target transcripts, while multiple miRNAs can regulate the same gene [37].

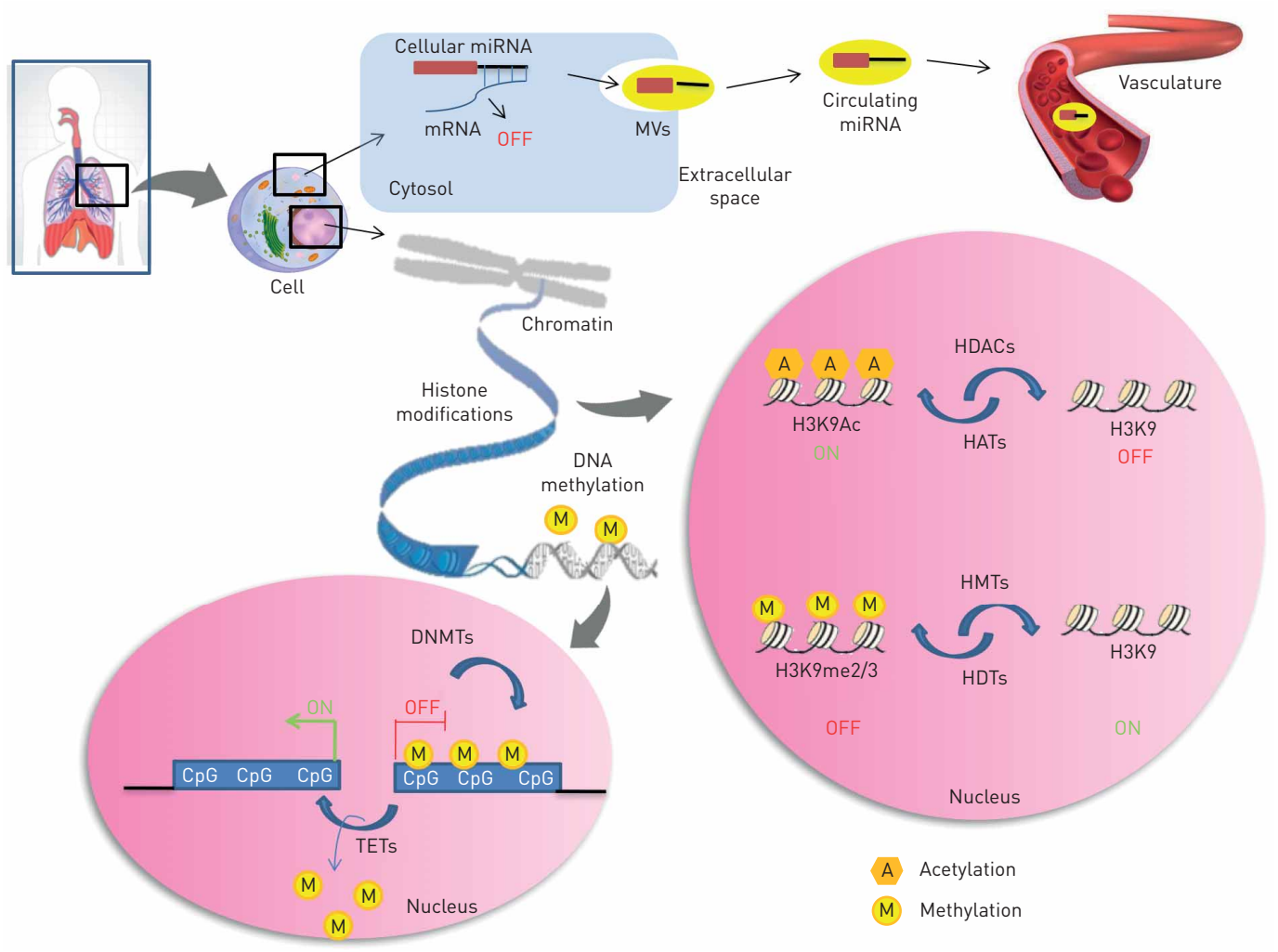


FIGURE 1 Basic epigenetic mechanisms. We illustrate the model of action of the three main epigenetic mechanisms including DNA methylation, histone modifications and microRNAs (miRNAs; see text for details). DNMT: DNA methyltransferase; HAT: histone acetyltransferase; HDAC: histone deacetyltransferase; HDT: histone demethyltransferase; HMT: histone methyltransferase; MV: microvesicle; TET: 10–11 translocation methylcytosine dioxygenase.

## Network medicine and pulmonary diseases

The pathogenesis of pulmonary diseases is triggered by complex interactions among heterogeneous molecular and environmental factors. Dissecting signalling pathways and molecular networks rather than single gene associations may allow us to clarify the aetiology and development of lung dysfunction. Here, we introduce the basic elements for understanding the framework of network-oriented analysis.

### *Defining molecular networks*

Nodes and edges form the key components of a network-oriented analysis applied to patient-derived omics data [13–16]. In biological networks, nodes refer to the molecular players, such as genes, proteins, metabolites and noncoding RNAs, while edges represent the functional relationships between a pair of connected nodes; for example PPIs, enzymatic reactions, transcriptional and transductional regulation, post-translational protein modifications, miRNA–long noncoding RNAs (and others) [13–16]. These molecular interactions may vary over time and space in response to environmental changes, providing "dynamic networks" [13–16]. Highly studied types of molecular networks include PPI networks, which are based on the human molecular interactome, and gene regulatory networks, which are based on transcription factor–DNA and protein–ligand binding [13–16]. In addition, co-expression networks are constructed to find modules of highly correlated genes based on their expression profiles (or co-methylation profiles) [13–16]. Biological networks are organised through quantifiable rules. For example, most biological networks have a topology that is "scale-free", *i.e.* the node distribution follows a power law [38]. From a functional point of view, network hubs (nodes with many connections) represent

the roots of connectivity among essential genes that can lead to embryonic lethality if mutated, whereas peripheral nodes (less highly connected nodes located far from the centre of the network) often represent genes harbouring the genetic variants that are responsible for phenotypic heterogeneity or that contribute the most to complex disease risk [13–16]. Thus, a network analysis can provide insights into the behaviour of complex biological systems and suggest putative useful clinical biomarkers or drug targets by identifying, for example, dysregulated hubs in the PPI network.

#### *Network analysis in transition from health to disease*

The "disease module hypothesis" states that the nodes and edges relevant to disease pathogenesis are more likely to be located in the same region, or "subnetwork", rather than being randomly scattered throughout the molecular interactome [13–16]. Many advanced network-oriented algorithms have been applied to patient-derived data to define the functional and mechanistic pathways underlying pulmonary diseases [4, 39–50]. These have generally included two main approaches: 1) identifying novel candidate genes localised in lung disease modules and 2) measuring the distance between the module in which the putative candidate gene is present and a known module for a disease [13–16].

Network analyses of lung diseases are currently often performed by analysing biological samples isolated from end-stage patients and control subjects. This approach only provides associative evidence between disease phenotype and molecular biomarkers and thus does not offer a temporal modelling of the dynamic molecular networks responsible for key transition events from health to disease. However, chronic respiratory disease pathogenesis is probably a multistage process moving from a healthy state toward a pre-disease state (reversible phase) which can transition to a disease state (irreversible or reversible phase). Thus, there is a need to perform analyses on the evolution of the relevant biological networks across healthy, pre-disease state (at risk, asymptomatic) and disease state subjects in order to pick up early network signals that initiate the critical transition to disease; these may point to useful biomarkers for primary prevention [51].

## The proposed hypothesis of "fetal origins" of pulmonary diseases: epigenetics for primary prevention?

The hypothesis supporting fetal origins for human disease arises from a convincing relationship between environmental exposures, lower birthweight and higher risk of cardiovascular dysfunction later in life [52]. Epigenetic-sensitive changes could bridge the mechanistic gap between many environmental and genetic risk factors providing a long-lasting memory of early detrimental exposures in COPD and asthma [18–20, 44], as well as in PAH [53]. DNA methylation plays a key role during fetal development; thus, large prospective birth cohorts have investigated DNA methylation as a surrogate for association between maternal [18–20] and/or paternal [19] exposures and risk of asthma and COPD in offspring. The largest meta-analysis of cord blood-based studies, including 1688 children from five cohorts revealed that specific differentially methylated regions (DMRs) were associated with childhood lung function, childhood asthma and COPD in adulthood. Among the top annotated genes, DMRs located in the homeobox A5 (*HOXA5*) gene promoter, involved in morphogenesis of fetal lung, correlated to childhood/adolescent forced expiratory volume in 1 s ($FEV_1$) and COPD development [18]. Moreover, hypermethylation of the promoter of the *SMAD3* (small mother against decapentaplegic 3) gene in neonatal life predicted the risk of asthma in childhood in those individuals born to asthmatic mothers [20]. Interestingly, a birth cohort of 1629 newborns revealed that the presence of a father with a smoking history was associated with persistent hypermethylation of immune master players, including the genes LIM domain only 2 (*LMO2*) and interleukin 10 (*IL-10*) and development of childhood asthma [19]. This evidence supports the hypothesis that maternal/paternal transgenerational effects can perturb biological processes in offspring *via* DNA methylation modifications. Thus, fetal epigenetic programming may influence COPD and asthma risk during adulthood. Methylome profiling at birth might eventually be a useful clinical tool to identify subjects with higher risk of pulmonary diseases and improve opportunities for primary prevention (figure 2). PAH has less support for epigenetic programming [5]. Although still debated in humans, fetal programming may impact epigenetic-sensitive pathways underlying the inheritance of pulmonary diseases and understanding these pathways may reveal new opportunities for primary and primordial prevention.

## Differential epigenetic biomarkers for prevention of pulmonary diseases in post-natal life

Here, we summarise results about putative epigenetic-sensitive biomarkers useful to measure the individual risk for pulmonary disease onset and progression (tables 1–3) [54–74]. We use these studies as exemplars of progress in the field toward the identification of epigenetic marks for further rigorous study in network analyses and functional validation. In addition, we summarise a list of studies from https://clinicaltrials.gov

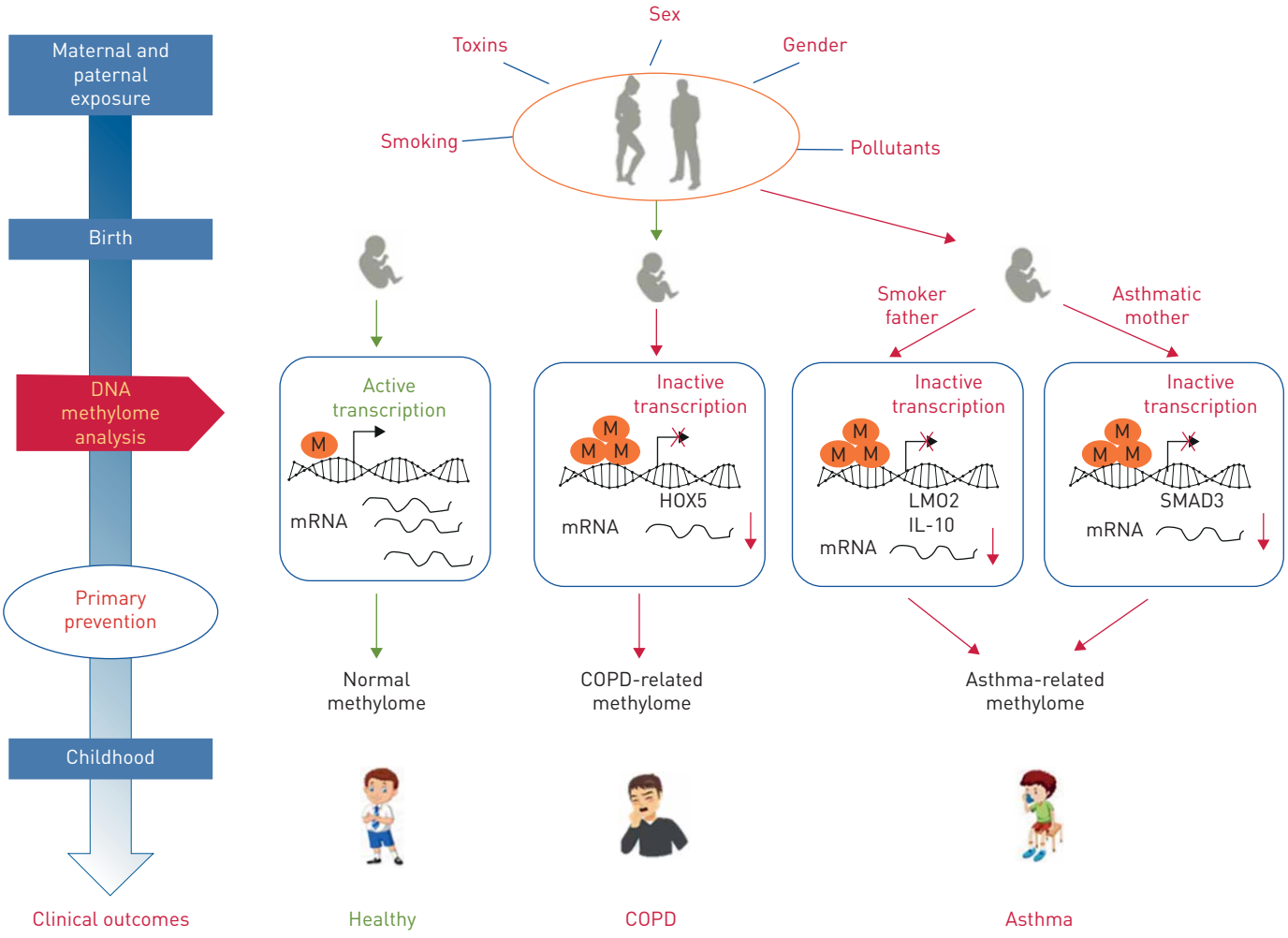


FIGURE 2 Epigenetic susceptibility to pulmonary diseases. DNA methylation changes impressed during fetal development may be inherited from parents (transgenerational effects) in addition to the genetic background contributing to development of pulmonary diseases later in life. Thus, the sex of the newborn would be relevant epigenetically as well as the contribution both of the mother and the father. DNA methylation analysis of target genes at birth, mainly hypermethylation of homeobox 5 (HOX5), LIM domain only 2 (LMO), interleukin 10 (IL-10) and mothers against decapentaplegic homologue 3 (SMAD3) might be useful to predict the risk of developing COPD and asthma.

and https://eudract.ema.europa.eu/ in supplementary table S1 to highlight the increasing interest in clinical epigenetics [75–78].

### *COPD*

COPD is characterised by progressive obstruction of airflow associated with emphysematous destruction of lung parenchyma, small airway fibrosis and destruction, and/or hypersecretion of mucus with chronic bronchitis [79]. Smoking is the most powerful risk factor for COPD development; as an exposure, smoking has been investigated in humans from *in utero* to old age and demonstrates extensive epigenetic effects and is a model of the impact of environmental exposure on the human genome. The epigenetic impact of smoking persists years after smoking cessation [80, 81]. However, some other inhaled noxious particles and gases, such as biomass combustion products, are also established COPD risk factors [79].

#### *DNA methylation*

There are many studies investigating the association between DNA methylation, COPD and lung function, mostly based on the analysis of circulating blood cells [54, 55] and less on lung tissue [56] providing putative useful biomarkers. An epigenome-wide association study (EWAS) of pulmonary function and COPD (274 COPD cases *versus* 2919 controls) based on peripheral blood samples identified 28 differentially methylated CpG sites associated with pulmonary function levels and/or COPD, mapping to genes involved in alternative splicing, JAK-STAT signalling and axon guidance; further research into DNA

TABLE 1 Summary of examples of DNA methylome approaches applied to the study of pulmonary diseases

| | Sample size | Sample source | NGS platform | Results | Ref. |
|---|---|---|---|---|---|
| **COPD** | 274 COPD cases and 2919 controls from the GS:SFHS | PBMCs | Infinium MethylationEPIC BeadChip and Infinium HumanMethylation450 BeadChip array | Differentially methylated CpG in genes linked to alternative splicing, and JAK-STAT signalling and axon guidance were associated with pulmonary function in COPD | [54] |
| | NAS retrospective discovery cohort (n=633) and KORA prospective replication cohort (n=868) | PBMCs | Infinium HumanMethylation450 BeadChip platform | *AHRR* and *IER3* genes correlated to metastable DNA methylation loci and decline of pulmonary function in COPD | [55] |
| | 114 patients (all former smokers) *versus* 46 healthy controls | Lung tissue samples | Infinium HumanMethylation450 BeadChip array | A correlation between methylation patterns of *PITPNM1* and *HNF1B* genes and their expression levels was observed | [56] |
| **Asthma** | 547 children from Project Viva study | Nasal swabs | MethylationEPIC BeadChip | DNA hypomethylation of *EPX* gene triggering Th2 and eosinophilic responses resulted associated with asthma and allergy phenotypes | [57] |
| | CAMP discovery set (n=154), BAMSE (n=72) and GACRS replication sets (n=168) | PBMCs | Infinium HumanMethylation27 BeadChip assay | DNA hypomethylation of *IL12B* and DNA hypermethylation of *CORT* genes may be useful predictive biomarkers of response to inhaled corticosteroid | [58] |
| | MRCA discovery cohort (n=355), PAPA (n=149) and SLSJ replication cohorts (n=160) | Eosinophils | HumanMethylation27 BeadChips | A correlation between serum IgE levels and hypomethylation of *IL5RA*, *IL1RL1*, *GATA1* encoding for eosinophil products and phospholipid inflammatory mediators was found | [59] |
| | Dutch PIAMA discovery cohort (n=455), EVA-PR (n=487) and ICAS (n=72) replication cohorts | Nasal epithelial brush | Infinium HumanMethylation450 BeadChips | DNA methylation profiles of many immune-related genes may be useful predictors of asthma and rhinitis in children | [60] |
| | 26 patients with persistent asthma, 39 remission patients and 70 healthy controls | Bronchial biopsies | Infinium HumanMethylation450 BeadChip array | *ACKR2* gene, involved in resolution of inflammation, may be a useful biomarker of asthma remission *versus* persistent phenotype | [61] |
| **PAH** | Idiopathic (n=11) and heritable (n=10) PAH patients *versus* 18 healthy controls | PAEC | Illumina HumanMethylation450 Assay | Hypermethylation of *ABCA1* gene suggested that dyslipidaemias closely correlated with PAH | [62] |
| | 12 HPAH patients and their unaffected relatives (controls) | PBMCs | Genomic bisulfite sequencing | Hypermethylation of *BMPR2* gene in wild-type alleles may be a putative regulatory mechanisms of low penetrance in PAH patients | [63] |

NGS: next-generation sequencing; PAH: pulmonary arterial hypertension; GS:SFHS: Generation Scotland Scottish Family Health Study; PBMCs: peripheral blood mononuclear cells; NAS: Normative Aging Study; KORA: Cooperative Health Research in the Region of Augsburg; AHRR: aryl-hydrocarbon receptor repressor; IER3: immediate early response 3; PITPNM1: phosphatidylinositol transfer protein, membrane associated 1; HNF1: HNF1 homeobox B; EPX: eosinophil peroxidase; Th: T-helper cell; CAMP: Childhood Asthma Management Program; BAMSE: Children Allergy Milieu Stockholm Epidemiology; GACRS: Genetic Epidemiology of Asthma in Costa Rica Study; IL12B: interleukin-12 subunit β; CORT: pre-cortistatin; IL5RA: interleukin 5 receptor subunit α; IL1RL1: interleukin 1 receptor like 1; GATA1: GATA binding protein 1; MRCA: Medical Research Council A; PAPA: Poblogaeth Asthma Prifysgol Abertawe; SLSJ: Saguenay-Lac-Saint-Jean region; PIAMA: Prevention and Incidence of Asthma and Mite Allergy; EVA-PR: Inner City Asthma Study and the Epigenetic Variation and Childhood Asthma in Puerto Ricans; ICAS: Inner-City Asthma Study; ACKR2: atypical chemokine receptor 2; PAEC: pulmonary arterial endothelial cells; ABCA1: ATP-binding cassette 1; HPAH: heritable PAH; BMPR2: bone morphogenetic protein receptor type 2.

methylation associations may improve prediction of COPD risk [54]. In addition, a publicly available dataset of COPD case–control lung tissue was retrieved to investigate the functional impact of DNA methylation changes on gene expression, suggesting an enrichment for JAK-STAT signalling genes

TABLE 2 Summary of examples of histone modifications associated with pulmonary diseases

| | Sample size | Sample source | Technique | Results | Ref. |
|---|---|---|---|---|---|
| **COPD** | | | | | |
| *Ex vivo* | 34 COPD patients (stage 1–4) *versus* 10 healthy controls | Bronchial epithelial cell biopsy | RT-qPCR, ChIP, bisulfite sequencing | Higher *DEFB1* expression was associated with increased H3K4me3 levels and pathological changes characteristic for COPD and disease severity | [64] |
| **Asthma** | | | | | |
| *Ex vivo* | 8 steroid-sensitive *versus* 11 steroid-resistant asthmatics patients | Monocytes | RT-qPCR, Western blot, ELISA, ChIP | Hyperacetylation of H4 at the *DUSP1* gene may mediate vitamin D anti-inflammatory and corticosteroid-enhancing effects in asthmatics | [65] |
| | 12 patients (mild to moderate) *versus* 12 healthy subject | Circulating $CD04^+$ T-cells | ChIP-seq, RNA sequencing | Increased levels of H3K4me2 mapped in silenced enhancers of Th2 from asthmatic patients supporting a pathogenic role in disease onset | [66] |
| **PAH** | | | | | |
| *In vitro* and *ex vivo* | 30 patients *versus* 26 failed donors | Lung tissue and PASMCs | RT-qPCR, Western blot, bisulfite sequencing, enzymatic activity | Decreased lung and PASMC SOD3 expression and activity were observed in PAH | [67] |
| *Ex vivo* | 10 PAH patients *versus* 7 controls | PAECs | Administration of siRNA | Selective inhibition of HDAC4 and 5 led to restoration of MEF2 function, suggesting a potential therapeutic role | [68] |

PAH: pulmonary arterial hypertension; RT-qPCR: reverse transcriptase quantitative PCR; ChIP: chromatin immunoprecipitation assay; DEFB1: β-defensin 1; H3K4me3: H3 lysine 4 tri-methylation; H4: histone 4; DUSP1: glucocorticoid response element upstream of the dual specificity phosphatase 1 gene; Th2: type 2 T-helper cell; PASMCs: pulmonary artery smooth muscle cells; SOD3: extracellular superoxide dismutase; PAEC: pulmonary artery endothelial cells; siRNA: small interfering RNA; HDAC: histone deacethylase; MEF2: myocyte enhancer factor 2.

including the suppressor of cytokine signalling 3 (*SOCS3*) [54]. This warrants further functional validation experiments in large cohorts to establish a possible causal relationship between methylation changes and COPD. A longitudinal EWAS identified targeted meta-stable DNA methylation changes at CpG dinucleotides in peripheral blood strongly associated with lung function decline and ageing in individuals from two independent cohorts (discovery set n=633; replication set n=868) [55]. Meta-stable methylation loci annotated to the aryl-hydrocarbon receptor repressor (*AHRR*) gene were associated with reduced $FEV_1$, forced expiratory flow at 25–75% of forced vital capacity and ageing, whereas the immediate early response 3 (*IER3*) gene was associated with reduced $FEV_1$, independent of ageing, suggesting a potential biomarker to predict lung function decline in the general population [55]. By combining genome-wide DNA methylation analysis of lung tissue samples from 114 COPD patients (all former smokers) *versus* 46 healthy controls with previous GWAS results, Morrow *et al.* [56] reported an enrichment for CpG differential methylation in shelves and shores annotated to several transcription factors, such as the forkhead box k1 (FOXK1) and FOXP2 transcription factors, as well as asthma-related genes, including the M1 muscarinic acetylcholine receptor (*CHRM1*) gene. However, there has been limited consistency between studies of the associations of lung function or COPD with differentially methylated sites, and one limiting factor may be related to the cellular heterogeneity of lung tissue and whole blood.

### *Histone modifications*

Few studies have focused on histone modifications to clarify the molecular basis of lung inflammation in COPD pathogenesis. Sundar *et al.* [82] demonstrated that cigarette smoke exposure triggered I κB kinase α (IKKα)-mediated phosphorylation (activation) of the mitogen- and stress-activated kinase 1 (MSK1) with consequent phospho-acetylation of histone H3 (Ser10/Lys9) and acetylation of histone H4 (Lys12) harboured at NF-κB-dependent promoters in H292, BEAS-2B and SAEC cells, leading to transcriptional activation of genes probably involved in COPD pathogenesis. Higher expression of β-defensin 1 (*DEFB1*) was associated with higher histone deacetylase 1 mRNA levels and correlated with poor lung function parameters in bronchopulmonary specimens of patients with COPD (n=34) *versus* healthy controls (n=10) [64].

### *Micro-RNAs*

miR-145 negatively regulated the release of pro-inflammatory cytokines, such as IL-6 and C-X-C motif chemokine ligand 8 (CXCL8), by targeting SMAD3 in airway smooth muscle cells isolated from nine COPD patients *versus* 18 healthy controls [69]. Interestingly, further administration of miR-145 mimics

TABLE 3 Summary of examples of microRNAs (miRNAs) associated with pulmonary diseases

| | Sample size | Sample source | Main techniques | Results | Ref. |
|---|---|---|---|---|---|
| **COPD** | | | | | |
| Cell-based assay | 9 COPD patients, 9 healthy nonsmokers, 9 healthy smokers | TGF-β-stimulated ASM | MicroRNA and messenger RNA expression, transfection with miR-145 mimics, Western blot | Administration of miR-145 mimics reduced IL-6 and CXCL8 expression in COPD ASM cells suggesting a putative drug targets | [69] |
| Tissue biopsy and cultured cells | 29 COPD patients with moderate emphysema *versus* mild emphysema | Lung tissue, BEAS-2B, HFL1 | RT-qPCR, transfection | Downregulation of miR-34b was associated with emphysema severity in COPD by modulating expression of SERPINE1 | [70] |
| **Asthma** | | | | | |
| Liquid-based assay | 153 asthmatic children | Serum | TaqMAN miRNA quantitative PCR primers | miR-146b, miR-206 and miR-720 which are involved in NF-κB and GSK3/AKT pathways might improve asthma exacerbation risk prediction | [71] |
| | Discovery set: 26 asthmatic patients *versus* 10 healthy subjects, validation set: 50 asthmatic patients *versus* 10 controls | Sputum supernatants | RT-qPCR | Upregulation of miR-629-3p, miR-223-3p, and miR-142-3p was significantly associated with severe asthma suggesting useful diagnostic biomarkers | [72] |
| **PAH** | | | | | |
| Cell-based assay | 19 PAH patients *versus* 13 healthy subjects | PASMCs, 293T and 293A | Transfection, RT-qPCR, Western blot, DNA methylation assay | Low levels of miR-1281 may have a possible diagnostic role | [73] |
| Tissue biopsy and cells | 130 PAH patients *versus* 91 healthy subjects | FFPE lung sections, PASMCs, PBMCs | Immunohistochemistry, siRNA treatment, microarray, *in silico* analysis, RT-qPCR | The miR-34a-3p-MiD axis promoted the Warburg effect *via* accelerating mitotic fission suggesting a useful biomarker and drug target for PAH | [74] |

PAH: pulmonary arterial hypertension; TGF: transforming growth factor; ASM: airway smooth muscle; miR: micro-RNAs; IL-6: interleukin-6; CXCL8: C-X-C motif chemokine ligand 8; BEAS-2B: human bronchial epithelial cell line; HFL1: human fetal lung fibroblast cell line; RT-qPCR: reverse transcriptase quantitative PCR; SERPINE1: serine proteinase inhibitor 1; PASMCs: pulmonary arterial smooth muscle cells; 293T: human embryonic kidney; 293A: 293T subclone; PBMCs: peripheral blood mononuclear cells; siRNA: small interfering RNA; MiD: mitochondrial dynamics protein.

reduced IL-6 and CXCL8 expression in COPD airway smooth muscle cells to levels comparable to controls, suggesting a novel putative drug therapy [69]. By using a microarray approach, Savarimuthu Francis *et al.* [70] found a significant downregulation of miR-34c, miR-34b, miR-149, miR-133a and miR-133b in lung tissue from 29 COPD patients with moderate emphysema *versus* mild emphysema undergoing resection for lung cancer. Among them, miR-34c showed the largest degree of association and modulated expression of its putative target mRNAs, such as the serpin family e member 1 (*SERPINE1*) gene.

### *Asthma*

Asthma is a characterised by chronic inflammation of the airways leading to respiratory symptoms including wheezing, shortness of breath, chest tightness, cough and variable airflow limitation, which vary over time and in intensity [83]. Asthma global prevalence ranges from 1% to 21% in adults and up to 20% of children aged 6–7 years [84].

#### *DNA methylation*

Recently, an EWAS conducted on genomic DNA from nasal epithelial cells of 547 children reported a strong correlation between elevated biomarkers of allergic disease, including fractional exhaled nitric oxide and total IgE, and differentially methylated regions that were annotated to genes involved in structure and function of epithelial cells, oxidative stress and mucin production enzymes in asthmatics *versus*

never-asthmatics [57]. A consistent signal arose from hypomethylation status at the eosinophil peroxidase (*EPX*) gene promoter which has been implicated in driving type 2 T-helper (Th2) cell and eosinophilic responses; however, there were no statistically significant associations between DNA methylation and lung function [57]. Furthermore, a DNA methylome analysis in peripheral blood mononuclear cells (PBMCs) from three independent cohorts (n=394 subjects) including mild-to-moderate asthmatic patients found that hypomethylation both of the IL-12 subunit β (*IL12B*) and precortistatin (*CORT*) genes correlated with decreased hospitalisation and oral corticosteroid use, respectively, suggesting useful prognostic biomarkers [58]. A large family-based EWAS on circulating eosinophils isolated from 95 European pedigrees demonstrated significant associations between serum IgE and methylation levels at 36 promoter regions annotated to genes coding for eosinophil products and phospholipid inflammatory mediators, specific transcription factors and mitochondrial proteins [59]. Hypomethylation of genes encoding eosinophil products was highly representative of asthmatics with high IgE *versus* asthmatics with low IgE levels and controls, suggesting potential risk stratification biomarkers [59]. More recently, an integrated methylome-single cell transcriptome analysis was performed by collecting nasal epithelial brush samples from 455 children (aged 16 years) with asthma, rhinitis, and asthma and rhinitis (AsRh) [60]. In these data, replicable differentially methylation sites were associated with the rhinitis and AsRh phenotypes (but not with asthma) and suggested useful disease predictors which were enriched for immune pathways, such as microglia pathogen phagocytosis pathway, DAP12 interactions, adaptive immune system, IL-2 signalling pathway and T-cell receptor signalling pathway [60]. Another multi-omics approach has investigated the impact of differential methylation on gene expression in bronchial biopsies from 26 persistent asthmatic patients and 39 asthma patients in remission *versus* 70 healthy controls [61]. In this study, four CpG-sites and 42 DMRs were found in persistent asthma *versus* the remission group. In particular, the most significant CpG site and DMR were hypomethylated in remission *versus* persistent asthma and associated with lower expression of the atypical chemokine receptor 2 (*ACKR2*) in subjects with remission [61]. The *ACKR2* gene mediates resolution of inflammation, suggesting a potential molecular mechanism involved in the remission of asthma.

#### *Histone modifications*

Some studies clarified the mechanisms of histone modulation controlling cytokine production and its role in the onset/severity of allergic diseases. A genome-wide mapping of histone modifications in $CD4^+$ memory, Th1 and Th2 cells from 12 asthmatic patients *versus* 12 healthy subjects revealed a differential enrichment of histone H3 lysine 4 di-methylated (H3K4me2) in Th2 enhancers associated with asthma susceptibility [66]. Moreover, a significant enrichment for binding sites of transcription factors involved in T-cell differentiation, such as GATA3, TBX21 and RUNX3, as well as an over-representation of genes involved in chemokine and Toll-like receptor signalling pathways, were observed [66]. In addition, hyperacetylation of histone H4 at the glucocorticoid response element upstream of the dual specificity phosphatase 1 gene (*DUSP1*), encoding for mitogen-activated protein kinase (MAPK) phosphatase 1 (MKP-1), was found in monocytes treated with dexamethasone obtained from eight steroid-sensitive and 11 steroid-resistant asthmatics patients. Additionally, vitamin D pre-incubation increased the dexamethasone-induced H4 acetylation, providing anti-inflammatory effects [65].

#### *Micro-RNAs*

A panel of circulating serum miRNAs, including miR-146b, miR-206 and miR-720, which are involved in NF-κB and GSK3/AKT pathways, might improve the accuracy of asthma exacerbation risk prediction in a paediatric asthma cohort (n=153) [71]. Indeed, the integrated miR-clinical score model showed high predictive power in discriminating exacerbation *versus* no exacerbation (area under the receiver operating characteristic curve 0.81) [71]. Maes *et al.* [72] investigated the possible association between miRNA expression in sputum with the inflammatory cell profile from 17 patients with mild-to-moderate asthma, nine patients with severe asthma and 10 healthy individuals. After validation in a second independent cohort, upregulation of miR-629-3p, miR-223-3p and miR-142-3p were significantly associated with severe neutrophilic asthma suggesting putative useful biomarkers to predict disease severity [72].

### *PAH*

PAH is a relatively rare cardiopulmonary disease characterised by irreversible vascular remodelling of pulmonary arterial walls leading to progressive vasoconstriction, inflammation, and thrombosis which may culminate in right ventricle failure [5, 84]. Vascular cells in PAH patients share some cancer hallmarks, such as hyperproliferation, resistance to apoptosis, migration and metabolic switch toward glycolysis (Warburg effect) by affecting epigenetic-sensitive pathways [5].

### *DNA methylation*

DNA methylation may play a role in PAH [5], but clinical data are limited. The first DNA methylome analysis of cultured pulmonary arterial endothelial cells (PAECs) isolated from 11 subjects with idiopathic PAH and 10 subjects with heritable PAH (HPAH) *versus* 18 healthy controls revealed that the most significant hypermethylated region mapped to the ATP-binding cassette 1 (*ABCA1*) promoter, suggesting that cholesterol metabolism is closely associated with PAH [62]. Moreover, the targeted bisulfite sequencing of DNA extracted from PBMCs revealed that the promoter region of the bone morphogenetic protein receptor type 2 (*BMPR2*) gene, which represents a relevant driver in PAH pathogenesis, was hypermethylated in the wild-type allele of 12 HPAH patients *versus* controls (unaffected relatives) suggesting a putative regulatory mechanism underlying the low penetrance of disease [63]. The next step is to perform integrative analysis which correlates methylome signatures in inflammatory circulating cells or disease-relevant tissue with quantitative gene expression to better understand PAH pathogenesis.

### *Histone modifications*

Histone acetylation imbalance is relevant in vascular remodelling of PAH patients, and the impaired gene expression and enzymatic activity of the extracellular superoxide dismutase (SOD3 or EC-SOD), a major vascular antioxidant enzyme, was related to activation of HDAC3 in lung tissue from end-stage PAH patients at time of lung transplantation *versus* lungs from failed donors [67]. The activity of the transcription factor myocyte enhancer factor 2 (MEF2), a key regulator of lung homeostasis, was significantly reduced in PAECs isolated from 10 PAH patients *versus* seven controls leading to lower levels of miR-424 and 503, connexins 37, and 40 and Krüppel-like factors 2 and 4 [68]. This detrimental effect was correlated to an excess of nuclear HDAC4 and HDAC5 activity. Moreover, treatment of PAECs with a small interfering RNA able to selectively target HDAC4 and HDAC5 led to reactivation of the MEF2 and expression of its gene targets, with associated decreased cell migration and proliferation, suggesting a therapeutic value in PAH [68].

### *Micro-RNAs*

Recently, a multiple epigenetic-sensitive regulatory axis, integrating phosphatidylinositol 3-kinase (PI3K)-DNMT1-miR-1281-HDAC4 signalling, has been implicated in proliferation and migration of pulmonary arterial smooth muscle cells (PASMCs) under platelet-derived growth factor stimulation [73]. In particular, low levels of miR-1281 were identified in 19 paediatric patients with PAH *versus* 13 healthy subjects, suggesting a possible diagnostic use [73]. Moreover, a strong correlation between low circulating miR-34a-3p levels and overexpression of mitochondrial dynamics protein of 49 and 51 kDa (MiD49 and MiD51, respectively) was found to play a role in promoting the Warburg effect in PASMCs from PAH patients *versus* controls, suggesting further useful biomarkers for disease treatment [74].

## Network approaches to modelling epigenetic variability in primary prevention of pulmonary diseases

Currently, the application of network analysis to epigenetic data is quite limited in pulmonary diseases; there has been greater focus on genetic variation affecting molecular mechanisms leading to disease phenotypes [40–43]. However, recent integrative omics studies have started to use network-oriented analyses to model interactions among biological, environmental and clinical features to develop novel hypotheses regarding lung disease mechanisms and to prioritise relationships for further validation. For example, network-oriented analyses have been applied to epigenetic data in fetal lung tissue exposed to *in utero* smoke and COPD [44], as well as in heterogeneous patient-derived cells or tissues in asthma [46, 47] and PAH [48, 49]. Weighted gene correlation network analysis (WGCNA) is a systems biology approach for identifying correlation patterns among the biological molecules measured in high-throughput experimental assays. WGCNA has been applied to DNA methylation data from fetal and adult lung tissue, revealing preserved network modules associated with fetal exposure to *in utero* smoke, COPD and lower adult lung function [44]. These modules were significantly enriched for genes involved in embryonic organ development and specific inflammation-related and ageing pathways, including Hippo, PI3K/AKT, Wnt, MAPK and transforming growth factor-β signalling, potentially supportive of the fetal origins of COPD [44]. Separately, a WGCNA analysis of leukocyte DNA methylation from 362 African American individuals with and without COPD identified comethylation modules associated with COPD that were enriched for genes related to inflammatory pathways, lung development and immune response, suggesting possible candidate genes contributing to racial differences in COPD susceptibility and severity [45]. In addition, by using linear regression and Pearson correlation analysis miRNA–mRNA pair co-expression networks were built, revealing that 20 miRNAs and 539 mRNAs were associated with chronic mucus hypersecretion (CMH) in bronchial biopsies of 63 COPD patients (from mild CMH to moderate/severe CMH) *versus* healthy controls belonging to the GLUCOLD study (NCT00158847) [46]. The gene set enrichment analysis and PPI-related STRING tool also predicted that upregulated miR-134-5p,

miR-146a-5p and the let-7 family and their potential target genes, including the KRAS proto-oncogene, GTPase (*KRAS*) and endothelin 1 (*EDN1*), may be key drivers of CMH in COPD [46]. Additionally, a molecular-bioinformatic approach applying WGCNA to DNA methylation and RNA sequencing data in primary airway epithelial cells from 74 asthmatic and 41 healthy subjects revealed that DNA methylation plays a relevant role in mediating the effects of individual genetic background on asthma risk and clinical outcome [47]. In addition, regulatory network analyses have provided important insights into pulmonary disease. For example, Reyes-Palomares *et al.* [48] built a multi-omic regulatory network of PAECs isolated from explanted lungs of 10 end-stage PAH patients and nine donor control individuals. Analysis of this network suggested that remodelling of active enhancers (marked by H3K27ac), rather than poised enhancers (marked by H3K4me1) and promoters (marked by H3K4me3), may prime PAH-PAECs towards the endothelial–mesenchymal transition without affecting gene expression [48]. Moreover, blood-based downregulation of miR-140-5p levels was found in treatment-naïve idiopathic PAH patients (n=4, female) compared to controls (n=4, female) [49]. Interestingly, network analysis predicted the SMAD-specific E3 ubiquitin protein ligase 1 (*SMURF1*) gene as a target for miR-140-5p target and a hub of bone morphogenetic protein (BMP) signalling [49]. This study also demonstrated that SMURF1 expression was increased in whole blood and remodelled pulmonary vasculature of PAH patients *versus* controls and that treatment with a miR-140-5p mimic may restore BMP signalling, suggesting a novel strategy to counteract onset and development of disease [49]. Furthermore, Parikh *et al.* [50] used miRNA relationships as filters to build a disease network module for PAH. Validation in PAECs revealed a key regulatory role for miR-21 upregulation in disease pathogenesis by impacting *BMPR2* gene expression.

## Novel perspectives

The "early life" stage, ranging from preconception (influenced by *in utero* exposures) through early childhood, is a critical temporal window during which detrimental insults may shape epigenetic signatures affecting fetal developmental and health later in life [8, 9, 18–20, 26, 44, 53]. Capturing tissue-specific epigenetic modifications by using network approaches might increase our knowledge about the fetal origins of diseases. The LifeCycle Project (https://lifecycle-project.eu/) is a European network of birth cohorts focusing on the role of early epigenetic-sensitive biomarkers, mainly DNA methylation, related to socioeconomic, migration, urban environment and lifestyle risk factors underlying pulmonary (and other) diseases. This consortium may be the starting point for investigating the possible impact of maternal and paternal lifestyle and nutritional habits on epigenetic-sensitive changes associated with pulmonary diseases by using a network-oriented strategy. This would have important implications for personalised diet and lifestyle interventions that may facilitate primary and primordial prevention of disease. Although the hypothesis of the fetal origins of diseases has gained international attention in recent years due to its potential in preventing complex diseases, there are still no definite strategies for implementation. Beyond obvious challenges from scientific and economic points of view, a critical barrier is the failure to communicate a key message to policymakers [85]. When elaborating on the possible implications of network medicine approaches for early life interventions for pulmonary diseases, two major questions should be clarified to the research community. First, how do we go from molecular networks to validated biomarker signatures to either predict disease risk or stratify patients for "individualised" therapies? Second, what is specifically needed to move from descriptive studies to actually implementing epigenetic biomarkers in clinical practice? Only future prospective randomised clinical trials will tell us whether specific early-life network-oriented epigenetic signatures may have a clinical utility in pulmonary disease management.

## Conclusions

Epigenetic-sensitive modifications have been associated with chronic lung diseases, suggesting a pathogenic role for epigenetic marks in disease onset and progression. Remarkably, DNA methylation may be inherited across several generations (transgenerational effect). This highlights the clinical potential for early-life methylome analysis in cord blood cells as a way to quantify the risk for disease onset, as observed for COPD and asthma, although the data are less compelling for PAH (figure 2). However, evidence for the role of cell-specific epigenetic marks in lung disease pathogenesis remains associative in the current clinical settings. In addition, it is still unknown whether most of the DMRs that are found in cord blood cells really reflect methylation in nasal cells or lung tissue. Therefore, these results cannot be used as predictors of disease in individuals, but are only useful from an aetiological perspective. In addition, DNA methylation patterns and expression of genes vary depending on the developmental stage [86] and are not be stable in the same individuals from birth to adulthood. Furthermore, DNA methylation marks are widely associated with sex [87–89] and age (epigenetic clock) [90–92] implying potential sex- and age-specific epigenomic responses to pre-natal exposures, which may serve as biomarkers of disease susceptibility. Thus, further integrative clinical studies should be performed to assess whether blood cells mirror the aberrant DNA methylation features and downstream modifications of gene

expression patterns in diseased lungs. By exploring molecular pathways, network-oriented algorithms have the potential to clarify the mechanistic links between exposures, epigenetic (and genetic) variability, and lung disease pathogenesis, offering novel opportunities for primary prevention and precision medicine. The involvement of specific pathways underlying the immune response seem to be an overlapping feature of COPD, asthma and PAH. Thus, it would be useful to further explore aberrant epigenetic features by multi-omics platforms and network-oriented analysis in selected circulating immune cells to detect molecular pathways which might be good signals to identify novel drug targets or for repurposing of "old drugs" (drug repurposing). However, the incomplete knowledge of the human interactome, the inaccuracy of gene ontology annotation, the lack of standard methods for validating network predictions, as well as the absence of randomised trials testing the clinical utility of network-based biomarkers, still limit the incorporation of network medicine to a bench approach in pulmonary precision medicine [5, 13, 15].

Author contributions: G. Benincasa, D.L. DeMeo, E.K. Silverman and C. Napoli contributed to the study concept and design. G. Benincasa, D.L. DeMeo, K. Glass, E.K. Silverman and C. Napoli contributed to acquisition, analysis, or interpretation of data. G. Benincasa, D.L. DeMeo and K. Glass drafted the manuscript. G. Benincasa, D.L. DeMeo and C. Napoli did critical revision of the manuscript for important intellectual content. G. Benincasa prepared the figures.

Conflict of interest: None declared.

Support statement: This manuscript was funded by National Research Competitive Grant (PRIN supporting grant no. F8ZB89) from Ministry for University and Research, Italy (to C. Napoli). G. Benincasa is a PhD Student in Translational Medicine supported from University of Campania "Luigi Vanvitelli," Naples, Italy. This study was also funded by US National Institutes of Health grants U01 HL089856, P01 HL114501, P01 HL132825, R01 HL147148, R01 HL133135, R01 HL137927 and K25 HL133599. E.K. Silverman reports institutional grant funding from GSK and Bayer. D.L. DeMeo reports institutional grant funding from Bayer and a research grant from the Alpha-1 Foundation. Funding information for this article has been deposited with the Crossref Funder Registry.

## References

1 Sakornsakolpat P, Prokopenko D, Lamontagne M, *et al.* Genetic landscape of chronic obstructive pulmonary disease identifies heterogeneous cell-type and phenotype associations. *Nat Genet* 2019; 51: 494–505.
2 Maselli DJ, Bhatt SP, Anzueto A, *et al.* Clinical epidemiology of COPD: insights from 10 years of the COPDGene Study. *Chest* 2019; 156: 228–238.
3 Rhodes CJ, Batai K, Bleda M, *et al.* Genetic determinants of risk in pulmonary arterial hypertension: international genome-wide association studies and meta-analysis. *Lancet Respir Med* 2019; 7: 227–238.
4 Hoang TT, Sikdar S, Xu CJ, *et al.* Epigenome-wide association study of DNA methylation and adult asthma in the Agricultural Lung Health Study. *Eur Respir J* 2020; 56: 2000217.
5 Napoli C, Benincasa G, Loscalzo J. Epigenetic inheritance underlying pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol* 2019; 39: 653–664.
6 Regan EA, Hersh CP, Castaldi PJ, *et al.* Omics and the search for blood biomarkers in chronic obstructive pulmonary disease. Insights from COPDGene. *Am J Respir Cell Mol Biol* 2019; 61: 143–149.
7 DeVries A, Vercelli D. Epigenetic mechanisms in asthma. *Ann Am Thorac Soc* 2016; 131: S48–S50.
8 Napoli C, Benincasa G, Donatelli F, *et al.* Precision medicine in distinct heart failure phenotypes: focus on clinical epigenetics. *Am Heart J* 2020; 224: 113–128.
9 Napoli C, Benincasa G, Schiano C, *et al.* Differential epigenetic factors in the prediction of cardiovascular risk in diabetic patients. *Eur Heart J Cardiovasc Pharmacother* 2020; 6: 239–247.
10 Loscalzo J, Handy DE. Epigenetic modifications: basic mechanisms and role in cardiovascular disease (2013 Grover Conference series). *Pulm Circ* 2014; 4: 169–174.
11 Napoli C, Crudele V, Soricelli A, *et al.* Primary prevention of atherosclerosis: a clinical challenge for the reversal of epigenetic mechanisms? *Circulation* 2012; 125: 2363–2373.
12 Napoli C, Infante T, Casamassimi A. Maternal–foetal epigenetic interactions in the beginning of cardiovascular damage. *Cardiovasc Res* 2011; 92: 367–374.
13 Benincasa G, Marfella R, Della Mura N, *et al.* Strengths and opportunities of network medicine in cardiovascular diseases. *Circ J* 2020; 84: 144–152.
14 Infante T, Del Viscovo L, De Rimini ML, *et al.* Network medicine: a clinical approach for precision medicine and personalized therapy in coronary heart disease. *J Atheroscler Thromb* 2020; 27: 279–302.
15 Silverman EK, Schmidt HHHW, Anastasiadou E, *et al.* Molecular networks in network medicine: development and applications. *Wiley Interdiscip Rev Syst Biol Med* 2020; 12: e1489.
16 Leopold JA, Maron BA, Loscalzo J. The application of big data to cardiovascular disease: paths to precision medicine. *J Clin Invest* 2020; 130: 29–38.
17 Meiners S, Eickelberg O, Königshoff M. Hallmarks of the ageing lung. *Eur Respir J* 2015; 45: 807–827.
18 den Dekker HT, Burrows K, Felix JF, *et al.* Newborn DNA-methylation, childhood lung function, and the risks of asthma and COPD across the life course. *Eur Respir J* 2019; 53: 1801795.
19 Wu CC, Hsu TY, Chang JC, *et al.* Paternal tobacco smoke correlated to offspring asthma and prenatal epigenetic programming. *Front Genet* 2019; 10: 471.
20 DeVries A, Wlasiuk G, Miller SJ, *et al.* Epigenome-wide analysis links SMAD3 methylation at birth to asthma in children of asthmatic mothers. *J Allergy Clin Immunol* 2017; 140: 534–542.
21 Feinberg AP. The key role of epigenetics in human disease prevention and mitigation. *N Engl J Med* 2018; 378: 1323–1334.
22 Reuben A, Sugden K, Arseneault L, *et al.* Association of neighborhood disadvantage in childhood with DNA methylation in young adulthood. *JAMA Netw Open* 2020; 3: e206095.

23 Dwi Putra SE, Reichetzeder C, Hasan AA, *et al.* Being born large for gestational age is associated with increased global placental DNA methylation. *Sci Rep* 2020; 10: 927.
24 Czamara D, Eraslan G, Page CM, *et al.* Integrated analysis of environmental and genetic influences on cord blood DNA methylation in new-borns. *Nat Commun* 2019; 10: 2548.
25 Wiklund P, Karhunen V, Richmond RC, *et al.* DNA methylation links prenatal smoking exposure to later life health outcomes in offspring. *Clin Epigenetics* 2019; 11: 97.
26 de Nigris F, Cacciatore F, Mancini FP, *et al.* Epigenetic hallmarks of fetal early atherosclerotic lesions in humans. *JAMA Cardiol* 2018; 3: 1184–1191.
27 Accordini S, Calciano L, Johannessen A, *et al.* A three-generation study on the association of tobacco smoking with asthma. *Int J Epidemiol* 2018; 47: 1106–1117.
28 Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. *Nat Rev Genet* 2009; 10: 295–304.
29 Selamat SA, Chung BS, Girard L, *et al.* Genome-scale analysis of DNA methylation in lung adenocarcinoma and integration with mRNA expression. *Genome Res* 2012; 22: 1197–1211.
30 Yoo S, Takikawa S, Geraghty P, *et al.* Integrative analysis of DNA methylation and gene expression data identifies EPAS1 as a key regulator of COPD. *PLoS Genet* 2015; 11: e1004898.
31 Tahiliani M, Koh KP, Shen Y, *et al.* Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 2009; 324: 930–935.
32 Fritz KS. Chemical acetylation and deacetylation. *Methods Mol Biol* 2013; 1077: 191–201.
33 Rea S, Eisenhaber F, O'Carroll D, *et al.* Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 2000; 406: 593–599.
34 Cruz C, Della Rosa M, Krueger C, *et al.* Tri-methylation of histone H3 lysine 4 facilitates gene expression in ageing cells. *Elife* 2018; 7: e34081.
35 Nguyen CT, Weisenberger DJ, Velicescu M, *et al.* Histone H3-lysine 9 methylation is associated with aberrant gene silencing in cancer cells and is rapidly reversed by 5-aza-2′-deoxycytidine. *Cancer Res* 2002; 62: 6456–6461.
36 Zhang T, Wu YC, Mullane P, *et al.* FUS regulates activity of microRNA-mediated gene silencing. *Mol Cell* 2018; 69: 787–801.
37 Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet* 2011; 12: 861–874.
38 Barabasi AL, Albert R. Emergence of scaling in random networks. *Science* 1999; 286: 509–512.
39 Samokhin AO, Stephens T, Wertheim BM, *et al.* NEDD9 targets *COL3A1* to promote endothelial fibrosis and pulmonary arterial hypertension. *Sci Transl Med* 2018; 10: eaap7294.
40 Sharma A, Menche J, Huang CC, *et al.* A disease module in the interactome explains disease heterogeneity, drug response and captures novel pathways and genes in asthma. *Hum Mol Genet* 2015; 24: 3005–3020.
41 Halu A, Liu S, Baek SH, *et al.* Exploring the cross-phenotype network region of disease modules reveals concordant and discordant pathways between chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *Hum Mol Genet* 2019; 28: 2352–2364.
42 Sharma A, Kitsak M, Cho MH, *et al.* Integration of molecular interactome and targeted interaction analysis to identify a COPD disease network module. *Sci Rep* 2018; 8: 14439.
43 Menche J, Sharma A, Cho MH, *et al.* A diVIsive Shuffling Approach (VIStA) for gene expression analysis to identify subtypes in chronic obstructive pulmonary disease. *BMC Syst Biol* 2014; 8: S8.
44 Kachroo P, Morrow JD, Kho AT, *et al.* Co-methylation analysis in lung tissue identifies pathways for fetal origins of COPD. *Eur Respir J* 2020; 56: 1902347.
45 Busch R, Qiu W, Lasky-Su J, *et al.* Differential DNA methylation marks and gene comethylation of COPD in African-Americans with COPD exacerbations. *Respir Res* 2016; 17: 143.
46 Tasena H, Faiz A, Timens W, *et al.* microRNA-mRNA regulatory networks underlying chronic mucus hypersecretion in COPD. *Eur Respir J* 2018; 52: 1701556.
47 Nicodemus-Johnson J, Myers RA, Sakabe NJ, *et al.* DNA methylation in lung cells is associated with asthma endotypes and genetic risk. *JCI Insight* 2016; 1: e90151.
48 Reyes-Palomares A, Gu M, Grubert F, *et al.* Remodeling of active endothelial enhancers is associated with aberrant gene-regulatory networks in pulmonary arterial hypertension. *Nat Commun* 2020; 11: 1673.
49 Rothman AM, Arnold ND, Pickworth JA, *et al.* MicroRNA-140-5p and SMURF1 regulate pulmonary arterial hypertension. *J Clin Invest* 2016; 126: 2495–2508.
50 Parikh VN, Jin RC, Rabello S, *et al.* MicroRNA-21 integrates pathogenic signaling to control pulmonary hypertension: results of a network bioinformatics approach. *Circulation* 2012; 125: 1520–1532.
51 Benincasa G, Mansueto G, Napoli C. Fluid-based assays and precision medicine of cardiovascular diseases: the 'hope' for Pandora's box? *J Clin Pathol* 2019; 72: 785–799.
52 Barker DJ. Fetal origins of coronary heart disease. *BMJ* 1995; 311: 171–174.
53 Jayet PY, Rimoldi SF, Stuber T, *et al.* Pulmonary and systemic vascular dysfunction in young offspring of mothers with preeclampsia. *Circulation* 2010; 122: 488–494.
54 Bermingham ML, Walker RM, Marioni RE, *et al.* Identification of novel differentially methylated sites with potential as clinical predictors of impaired respiratory function and COPD. *EBioMedicine* 2019; 43: 576–586.
55 Carmona JJ, Barfield RT, Panni T, *et al.* Metastable DNA methylation sites associated with longitudinal lung function decline and aging in humans: an epigenome-wide study in the NAS and KORA cohorts. *Epigenetics* 2018; 13: 1039–1055.
56 Morrow JD, Cho MH, Hersh CP, *et al.* DNA methylation profiling in human lung tissue identifies genes associated with COPD. *Epigenetics* 2016; 11: 730–739.
57 Cardenas A, Sordillo JE, Rifas-Shiman SL, *et al.* The nasal methylome as a biomarker of asthma and airway inflammation in children. *Nat Commun* 2019; 10: 3095.
58 Wang AL, Gruzieva O, Qiu W, *et al.* DNA methylation is associated with inhaled corticosteroid response in persistent childhood asthmatics. *Clin Exp Allergy* 2019; 49: 1225–1234.
59 Liang L, Willis-Owen SAG, Laprise C, *et al.* An epigenome-wide association study of total serum immunoglobulin E concentration. *Nature* 2015; 520: 670–674.
60 Qi C, Jiang Y, Yang IV, *et al.* Nasal DNA methylation profiling of asthma and rhinitis. *J Allergy Clin Immunol* 2020; 145: 1655–1663.

61 Vermeulen CJ, Xu CJ, Vonk JM, *et al.* Differential DNA methylation in bronchial biopsies between persistent asthma and asthma in remission. *Eur Respir J* 2020; 55: 1901280.
62 Hautefort A, Chesné J, Preussner J, *et al.* Pulmonary endothelial cell DNA methylation signature in pulmonary arterial hypertension. *Oncotarget* 2017; 8: 52995–53016.
63 Liu D, Yan Y, Chen JW, *et al.* Hypermethylation of BMPR2 promoter occurs in patients with heritable pulmonary arterial hypertension and inhibits BMPR2 expression. *Am J Respir Crit Care Med* 2017; 196: 925–928.
64 Andresen E, Günther G, Bullwinkel J, *et al.* Increased expression of beta-defensin 1 (DEFB1) in chronic obstructive pulmonary disease. *PLoS One* 2011; 6: e21898.
65 Zhang Y, Leung DY, Goleva E. Anti-inflammatory and corticosteroid-enhancing actions of vitamin D in monocytes of patients with steroid-resistant and those with steroid-sensitive asthma. *J Allergy Clin Immunol* 2014; 133: 1744–1752.
66 Seumois G, Chavez L, Gerasimova A, *et al.* Epigenomic analysis of primary human T cells reveals enhancers associated with TH2 memory cell differentiation and asthma susceptibility. *Nat Immunol* 2014; 15: 777–788.
67 Nozik-Grayck E, Woods C, Stearman RS, *et al.* Histone deacetylation contributes to low extracellular superoxide dismutase expression in human idiopathic pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol* 2016; 311: L124–L134.
68 Kim J, Hwangbo C, Hu X, *et al.* Restoration of impaired endothelial myocyte enhancer factor 2 function rescues pulmonary arterial hypertension. *Circulation* 2015; 131: 190–199.
69 O'Leary L, Sevinç K, Papazoglou IM, *et al.* Airway smooth muscle inflammation is regulated by microRNA-145 in COPD. *FEBS Lett* 2016; 590: 1324–1334.
70 Savarimuthu Francis SM, Davidson MR, Tan ME, *et al.* MicroRNA-34c is associated with emphysema severity and modulates SERPINE1 expression. *BMC Genomics* 2014; 15: 88.
71 Kho AT, McGeachie MJ, Moore KG, *et al.* Circulating microRNAs and prediction of asthma exacerbation in childhood asthma. *Respir Res* 2018; 19: 128.
72 Maes T, Cobos FA, Schleich F, *et al.* Asthma inflammatory phenotypes show differential microRNA expression in sputum. *J Allergy Clin Immunol* 2016; 137: 1433–1446.
73 Li Y, Li L, Qian Z, *et al.* Phosphatidylinositol 3-kinase-DNA methyltransferase 1-miR-1281-histone deacetylase 4 regulatory axis mediates platelet-derived growth factor-induced proliferation and migration of pulmonary artery smooth muscle cells. *J Am Heart Assoc* 2018; 7: e007572.
74 Chen KH, Dasgupta A, Lin J, *et al.* Epigenetic dysregulation of the dynamin-related protein 1 binding partners MiD49 and MiD51 increases mitotic mitochondrial fission and promotes pulmonary arterial hypertension: mechanistic and therapeutic implications. *Circulation* 2018; 138: 287–304.
75 Yang IV, Pedersen BS, Liu A, *et al.* DNA methylation and childhood asthma in the inner city. *J Allergy Clin Immunol* 2015; 136: 69–80.
76 Stolzenburg LR, Wachtel S, Dang H, *et al.* miR-1343 attenuates pathways of fibrosis by targeting the TGF-β receptors. *Biochem J* 2016; 473: 245–256.
77 Martin F, Talikka M, Hoeng J, *et al.* Identification of gene expression signature for cigarette smoke exposure response – from man to mouse. *Hum Exp Toxicol* 2015; 34: 1200–1211.
78 Han Y, Forfia PR, Vaidya A, *et al.* Rationale and design of the ranolazine PH-RV study: a multicentred randomised and placebo-controlled study of ranolazine to improve RV function in patients with non-group 2 pulmonary hypertension. *Open Heart* 2018; 5: e000736.
79 Singh D, Agusti A, Anzueto A, *et al.* Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease: the GOLD science committee report 2019. *Eur Respir J* 2019; 53: 1900164.
80 Joehanes R, Just AC, Marioni RE, *et al.* Epigenetic signatures of cigarette smoking. *Circ Cardiovasc Genet* 2016; 9: 436–447.
81 Wan ES, Qiu W, Baccarelli A, *et al.* Cigarette smoking behaviors and time since quitting are associated with differential DNA methylation across the human genome. *Hum Mol Genet* 2012; 21: 3073–3080.
82 Sundar IK, Chung S, Hwang JW, *et al.* Mitogen- and stress-activated kinase 1 (MSK1) regulates cigarette smoke-induced histone modifications on NF-κB-dependent genes. *PLoS One* 2012; 7: e31378.
83 Reddel HK, Bateman ED, Becker A, *et al.* A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J* 2015; 46: 622–639.
84 Maron BA, Wertheim BM. Toward early diagnosis of pulmonary hypertension: lessons from Oz. *J Am Coll Cardiol* 2019; 73: 2673–2675.
85 Jacob CM, Hanson M. Implications of the developmental origins of health and disease concept for policy-making. *Curr Opin Endocr Metab Res* 2020; 13: 20–27.
86 Li S, Zhu Y, Zhi L, *et al.* DNA methylation variation trends during the embryonic development of chicken. *PLoS One* 2016; 11: e0159230.
87 Curtis SW, Gerkowicz SA, Cobb DO, *et al.* Sex-specific DNA methylation differences in people exposed to polybrominated biphenyl. *Epigenomics* 2020; 12: 757–770.
88 Leung YK, Ouyang B, Niu L, *et al.* Identification of sex-specific DNA methylation changes driven by specific chemicals in cord blood in a Faroese birth cohort. *Epigenetics* 2018; 13: 290–300.
89 Hall E, Volkov P, Dayeh T, *et al.* Sex differences in the genome-wide DNA methylation pattern and impact on gene expression, microRNA levels and insulin secretion in human pancreatic islets. *Genome Biol* 2014; 15: 522.
90 Tajuddin SM, Hernandez DG, Chen BH, *et al.* Novel age-associated DNA methylation changes and epigenetic age acceleration in middle-aged African Americans and whites. *Clin Epigenetics* 2019; 11: 119.
91 McEwen LM, Jones MJ, Lin DTS, *et al.* Systematic evaluation of DNA methylation age estimation with common preprocessing methods and the Infinium MethylationEPIC BeadChip array. *Clin Epigenetics* 2018; 10: 123.
92 Markunas CA, Wilcox AJ, Xu Z, *et al.* Maternal age at delivery is associated with an epigenetic signature in both newborns and adults. *PLoS One* 2016; 11: e0156361.