The application of ‘omics’ to pulmonary arterial hypertension

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Recent genome-wide analyses of rare and common sequence variations have brought greater clarity to the genetic architecture of pulmonary arterial hypertension and implicated novel genes in disease development. Transcriptional signatures have been reported in whole lung tissue, pulmonary vascular cells and peripheral circulating cells. High-throughput platforms for plasma proteomics and metabolomics have identified novel biomarkers associated with clinical outcomes and provided molecular instruments for risk assessment. There are methodological challenges to integrating these datasets, coupled to statistical power limitations inherent to the study of a rare disease, but the expectation is that this approach will reveal novel druggable targets and biomarkers that will open the way to personalized medicine. Here, we review the current state-of-the-art and future promise of ‘omics’ in the field of translational medicine in pulmonary arterial hypertension.

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**1 | INTRODUCTION**

The last three decades have seen significant advances in the treatment of pulmonary hypertension (PH). Nonetheless, patients usually present late in the course of their illness with advanced symptoms and long-term survival is poor; there is still an unmet clinical need for better medicines (https://digital.nhs.uk/data-and-information/clinical-audits-and-registries/national-pulmonary-hypertension-audit). The search is on for a disease modifying therapy and this is dependent upon a deeper understanding of the pathology of pulmonary vascular disease, particularly in its early stages.

A major difficulty has been access to diseased tissue, as lung biopsies are too hazardous to acquire. The fall in costs of gene sequencing and increasing access to several modalities of high-throughput measurements of large numbers of molecules of a biological class, the so-called omics approach, has opened up new opportunities for patient-orientated research. Proteins, metabolites and various forms of RNAs that circulate in blood offer a ‘liquid biopsy’ of the large surface area

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**Abbreviations:** ABCA1, ATP binding cassette subfamily A member 1; ACVR1, activin A receptor type I; AQP1, aquaporin-1; Arf6, ADP ribosylation factor 6; ATP13A3, ATPase polyamine transporter; BMP, bone morphogenetic protein; BMPR2, BMP receptor type II; CAV1, caveola; Chip-seq, chromatin immunoprecipitation DNA sequencing; circRNA, circular RNA; CLIC4, chloride intracellular channel 4; ENG, endoglin; eQTL, expression QTL; FADH2, flavin adenine dinucleotide; GDF2, growth differentiation factor 2; GNG2, G protein subunit gamma 2; GWAS, genome-wide association study; HDACs, histone deacetylases; HLA, human leukocyte antigen; hm5C, 5-hydroxymethylcytosine; iPS, induced pluripotent stem; KCNK3, potassium two pore domain subfamily K member 3; lncRNA, long noncoding RNA; m1A, N¹-methyladenosine; m6A, N6-methyladenosine; m6Am, N6,2'-O-dimethyladenosine; METTL3, methyltransferase like 3; mQTL, quantitative trait locus; MR, Mendelian randomization; NADH, nicotinamide adenine dinucleotide; OLR1, oxidized LDL receptor 1; PAH, pulmonary arterial hypertension; PBMCs, peripheral blood mononuclear cells; PCR, quantitative trait locus; REVEAL, registry to evaluate early and long-term PAH disease management; SMAD, mothers against decapentaplegic homologues; sncRNA, short noncoding RNA; SOX17, SRY-box containing gene 17; TBX4, T-box transcription factor 4; TCA, tricarboxylic acid; TIMP, tissue inhibitor of metalloproteinase.

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of the diseased pulmonary vasculature. In addition to informing diagnosis and enabling disease monitoring, the integration of circulating 'omics' with genotypes and epigenetic modifications offers a route map for improving the taxonomy of PH according to molecular profiles, the definition of disease pathways and identifying novel druggable targets more relevant to the human pathology (Figure 1).

This approach is most advanced with pulmonary arterial hypertension (PAH). The sample size required for robust conclusions in this rare condition has led to the development of national and international networks, such as the PH Breakthrough Initiative (PHBI), the Pulmonary Arterial Hypertension Biobank and, more recently, the pulmonary vascular disease omics study (PVDOMICS) in the United States, the French network and the U.K. National Cohort Study of PAH (www.ipahcohort.com) in Europe, as well as international collaborations, such as the international PAH genetics consortium (www.pahicon.com). It is the number of subjects, rather than the acquisition of 'big data', that is the main factor limiting power.

2 | RECENT PROGRESS IN THE GENETICS AND GENOMICS OF PULMONARY ARTERIAL HYPERTENSION (PAH)

Since the first reports of heterozygous germline mutations in the bone morphogenetic protein receptor type 2, BMPR2, the list of genes associated with PAH has grown, such that between 20% and 30% of patients with idiopathic PAH are recognized to have an underlying Mendelian cause (Evans et al., 2016; Southgate, Machado, Graf, & Morrell, 2020). Mutations in BMPR2 are the most common, found in 70% to 80% of patients with a family history of PAH and 10% to 20% of patients with idiopathic PAH. Genetic studies of 'BMPR2-negative' families and, more recently, whole exome and whole genome sequencing of carefully curated patient cohorts have implicated a number of other genes harbouring rare variants (Table 1). Several of these (GDF2, ACVRL1, ENG, SMAD9, SMAD1 and SMAD4) lie within the BMPR2/TGF-β signalling pathway, emphasizing the pathological relevance of this pathway to pulmonary vascular homeostasis, but mutations in genes outside this cascade point to molecular heterogeneity in the pathology of PAH (Southgate et al., 2020). These include genes encoding a potassium channel (KCNK3), a transcription factor (TBX4), an endodermal transcription factor (SOX17), an ATPase polyamine transporter (ATP13A3), a scaffolding protein in caveolae (CAV1) and aquaporin-1 (AQP1; Graf et al., 2018). Additional candidates continue to be proposed (Eyries et al., 2020; Zhu et al., 2019). In silico tests supported by functional studies in model systems help adjudicate on the pathogenic significance of rare variants, particularly challenging when these are miss-sense or occur in noncoding regions. Evidence of familial segregation, where available, provides greater assurance.

Common genetic variant analysis using data from four international case–control studies across 11,744 individuals with European ancestry (including 2,085 patients) has identified a locus overlapping HLA-DPB1 that is associated with both the development of PAH and clinical outcomes (Rhodes et al., 2019). Polymorphisms in a putative enhancer upstream of SOX17 (which encodes SRY-box 17, Sox17) imply that perturbation of Sox17 activity may have a greater role in PAH than rare variation of SOX17 alone suggests. Sox17 is a member of the high mobility group transcription factor superfamily and targeted loss of Sox17 in the cells which form the pulmonary vasculature leads to enlarged pulmonary arteries, reduced perfusion of the distal lung and biventricular cardiac hypertrophy (Lange et al., 2014). Interestingly, rare SOX17 variants are greatly enriched (present in 3.2% of cases) in juvenile cases of PAH associated with congenital heart defects (Zhu et al., 2018). These discoveries are already having an impact on clinical practice. Gene panels are now available for genetic testing of affected individuals to inform family counselling; experience of best practice is available, supported by educational videos (https://www.youtube.com/watch?v=36rlvtj_Qrs).

So far, attention has been directed at documenting single-nucleotide variants, but increasingly, attention will turn to larger structural variation, including multi-copy-number variants and epigenetic modifications, such as methylation and histone acetylation patterns. Epigenetic alterations change gene expression without altering DNA sequence and are considered to have a significant role
| Reference                | Year of publication | Omics     | Sample type               | Diagnosis     | Case/Control | Main phenotype                  | Technique                      |
|-------------------------|---------------------|-----------|---------------------------|---------------|--------------|---------------------------------|--------------------------------|
| Germain et al., 2013    | 2013                | Genome    | Peripheral blood cells    | I/HPAH        | 625/1,525    | Case–control status             | Microarray                     |
| Graf et al., 2018       | 2018                | Genome    | Peripheral blood cells    | I/HPAH        | 1,038/6,385  | Case–control status             | WGS                            |
| Zhu et al., 2019        | 2019                | Genome    | Peripheral blood cells    | PAH           | 1,832/7,509  | Case–control status             | WES                            |
| Rhodes et al., 2019     | 2019                | Genome    | Peripheral blood cells    | I/HPAH        | 2,085/9,659  | Case–control status             | WGS, microarray                |
| Hautefort et al., 2017  | 2017                | Epigenome | Cultured PAEC             | I/HPAH        | 21/18        | Case–control status             | Microarray                     |
| Bull et al., 2004       | 2004                | Transcriptome | Peripheral blood cells | PAH           | 15/6         | Case–control status             | Microarray                     |
| Hautefort et al., 2017  | 2017                | Transcriptome | Laser-microdissected pulmonary arteries | I/PAH | 6/6 | Case–control status | Microarray |
| Cheadle et al., 2012    | 2012                | Transcriptome | Peripheral blood cells | PAH | 99/41 | Case–control status | Microarray |
| Hennes et al., 2015     | 2015                | Transcriptome | Cultured peripheral lymphocytes | I/HPAH | 40/– | Vasoresponder status | Microarray |
| Stearman et al., 2019   | 2019                | Transcriptome | Whole lung tissue         | PAH           | 58/25        | Case–control status             | Microarray                     |
| Abdul-Salam et al., 2010| 2010               | Proteome   | Whole lung tissue         | I/HPAH        | 8/8          | Case–control status             | LC–MS/MS                       |
| Rhodes, Wharton, et al., 2017 | 2017        | Proteome   | Plasma                     | I/HPAH        | 354/–        | Survival status                 | Modified-aptamer-based assay   |
| Sweatt et al., 2019     | 2019                | Proteome   | Plasma                     | PAH           | 385/–        | Immunophenotype                 | Magnetic bead-based antibody assay |
| Xu et al., 2019         | 2019                | Proteome   | Cultured PAEC             | PAH           | 4/5          | Case–control status             | LC–MS/MS                       |
| Zhao et al., 2014       | 2014                | Metabolome | Whole lung tissue         | PAH           | 8/8          | Case–control status             | LC–MS/MS                       |
| Lewis et al., 2016      | 2016                | Metabolome | Plasma                     | Suspected PH, PAH | 172/– | Pulmonary haemodynamics             | LC–MS                         |
| Rhodes, Ghataorhe, et al., 2017 | 2017        | Metabolome | Plasma                     | I/HPAH        | 365/260      | Case–control and survival status | MS                             |
| Harbaum et al., 2019    | 2019                | Metabolome | Plasma                     | I/HPAH        | 204/–        | Survival status                 | NMR                            |
in the interaction between genes and environment in the development of PAH (Figure 2; Napoli, Benincasa, & Loscalzo, 2019). Annotations of epigenetic markers are commonly cell/tissue-type specific and international efforts such as Encode, Roadmap and Blue-Print/International Human Epigenome Consortium have provided publicly available resources for studying genomic regulation (Kundaje et al., 2015; Stunnenberg & Hirst, 2016; Thurman et al., 2012). These data include a few primary human (healthy) pulmonary artery cells such as endothelial cells and fibroblasts (Thurman et al., 2012). In pulmonary endothelial cells harvested from PAH patients, a genome-wide methylation analysis revealed that promoter regions of genes encoding different lipid transporters, including ABCA1, were differently methylated (Hautefort et al., 2017). Targeting the proteins catalysing chromatin alterations represents a potential therapeutic strategy for PAH. Histone acetylation/deacetylation balance is controlled by two sets of enzymes, histone acetyltransferases and histone deacetylases (HDACs). HDACs remove acetyl groups from histones (and other nuclear proteins) and are up-regulated in lungs from PAH patients (Zhao et al., 2012). Early studies suggest that HDAC inhibition is able to reverse PH in animal models and human pulmonary vascular cells in culture (Zhao et al., 2012).

The expectation is that genetic and genomic discoveries will inform new drug development, not only by identifying and prioritizing novel drug targets but also through the design and interpretation of clinical trials. Translating these expectations into clinical reality is dependent upon integrating information from the genome with ‘omics’ data from other platforms and detailed clinical phenotyping.

3 | GENE EXPRESSION SIGNATURES IN PH

Oligonucleotide microarrays and, more recently, RNAseq enable the characterization of gene expression profiles in cells and tissues (Perrino et al., 2017). New technologies, such as nanopore sequencing, are emerging (Soneson et al., 2019). Genes can be ranked according to fold-change or differences in levels between samples and analysed for co-expression patterns that define pathways to counter reliance on chance variation in single genes (Hoffmann, Wilhelm, Olschewski, & Kwapiszewska, 2016). Access to appropriate cell types during the most informative stages of PAH is a major limiting factor. There is a dependence on tissue samples from explanted lungs, with the attendant confounding factors of end-stage disease and concomitant medication. Peripheral blood mononuclear cells (PBMCs) offer a more accessible alternative but their relevance to the diseased tissue can be questioned.

Whole lung tissue specimens in PAH are obtained mainly from lung transplantation. An early study of whole lung tissue from six patients with PAH, including two with heritable PAH, and six patients with histologically normal lungs, used oligonucleotide microarrays to reveal differences in gene expression patterns between PAH and normal lungs, and also between idiopathic PAH and heritable PAH (Geraci, Moore, et al., 2001; Stearman et al., 2019). The number of patients included has grown to over 50, allowing more comprehensive bioinformatic approaches, such as gene co-expression networks (Stearman et al., 2019); the multicentre PHBI gene expression dataset comprised 58 patient lung samples, including different PAH subtypes and 25 controls (Table 1; Stearman et al., 2019). These studies have highlighted an important role for TNF and TGF signalling pathways in PAH lungs (Stearman et al., 2019). Studies have been extended to other presentations of PH, revealing changes in the expression of genes involved in cell proliferation, inflammation, immunity and extra-cellular matrix turnover in PAH, consistent with the histology of the condition (Hsu et al., 2011; Mura et al., 2012; Rajkumar et al., 2010).

It has been argued that a fragment of lung tissue is representative of the whole lung as the vascular lesions are widely distributed throughout the entire lung (Geraci, Gao, et al., 2001), but there is heterogeneity in disease within the PAH lung. Analysis of laser-capture micro-dissected arteries provides a more targeted analysis and studies show perturbation of Wnt signalling in diseased vessels (Laumanns et al., 2009). Analysis of isolated primary vascular cells takes the targeted tissue approach further, at the expense of factoring in the contribution of non-vascular cells (e.g. inflammatory cells) to PAH (Rhodes et al., 2015). With that caveat, differences in gene expression profiles in human pulmonary artery endothelial cells and smooth muscle cells derived from idiopathic PAH and control subjects support down-regulation of BMPR2 signalling, even in the absence of BMPR2 mutations, as well as other factors regulating cell proliferation and survival (Rhodes et al., 2015).

Comparing diseased and healthy tissue or cells in isolation acts as a useful reference source for interpreting genomic variation but does not provide a robust platform for prioritizing pathways for developing therapeutic strategies. Microarray or RNAseq analysis of cells with

FIGURE 2 | Biological layout of a full ‘omics’ approach
known variants stimulated in vitro can assist with understanding signalling pathways. The use of induced pluripotent cells (iPS cells) derived from PAH patients with known mutations directed to differentiate into specific vascular subtypes (Gu et al., 2017) and the manipulation of iPS cells to create bespoke variants on an isogenic background (Kiskin et al., 2018) that can then be stimulated with candidate agonists is a particularly elegant and informative option.

Transcriptional analysis of peripheral blood mononuclear cells offers an opportunity for repeated sampling and has attracted interest for informing diagnosis and tailoring treatment (personalized medicine). There is robust evidence supporting the ability of peripheral blood to accurately reflect subtle changes resulting from injuries or disease processes, given the constant interactions between the different organs and peripheral blood mononuclear cells, which trigger changes in peripheral blood mononuclear cell transcriptional and phenotypical state (Mohr & Liew, 2007). That said, while studies have reported differences in gene expression in peripheral blood mononuclear cells from PAH and healthy individuals, we are still some distance from identifying a robust signature that distinguishes between the different presentations of PH, which would be helpful in clinical management (Bull et al., 2004; Cheadle et al., 2012; Chesne et al., 2014; Elinoff et al., 2020; Grigoryev et al., 2008; Pendergrass et al., 2010; Risbano et al., 2010). A gene expression signature that identified patients with idiopathic PAH likely to respond to vasodilator therapy has been reported (Hemnes et al., 2015), although the changes identified in the Wnt/PCP/Rho pathway are biologically credible and have been validated by qPCR, the signature remains to be confirmed in a prospective setting.

Internal RNA modification through methylation (also known as epitranscriptomics) and alternative splicing offers another level of regulating gene expression (Roundtree, Evans, Pan, & He, 2017). Methylation of adenosine (N6-methyladenosine, m6A) is well recognized, and transcriptome-wide mapping studies have identified specific gene categories that are associated with transcripts containing a disproportional high level of m6A including genes involved in organogenesis (Dominissini et al., 2012). The m6A modification of mRNA is catalysed by a set of proteins, including m6A methyltransferases (e.g. METTL3) and demethylases (e.g. fat mass and obesity associated), which are possible candidate drug targets in cardiovascular diseases (Dorn et al., 2019). Other chemical modifications of mRNA include N4-methyladenosine (m4A) and N6,2'-O-dimethyladenosine (m6Am), as well as cytosine methylation to 5-methylcytosine and its oxidation product 5-hydroxymethylcytosine (hm5C; Roundtree et al., 2017). To date, these have not been explored in PAH.

miRNAs are conserved, non-protein-coding RNA molecules that regulate gene expression by binding to the 3' untranslated regions of mRNA transcripts to repress translation and/or degrade mRNA. miRNAs have been implicated in cellular processes relevant to PAH, such as inflammation (Brock et al., 2009), TGF/BMP signalling (Brock et al., 2014; Calvier, Chouvarine, Legchenko, & Hansmann, 2017) and energy metabolism (Caruso et al., 2017; Zhang et al., 2017). A number of candidate miRNAs have been identified in isolated primary pulmonary vascular cells by microarray or sequencing methods, for example miR-424 and miR-503, which were discovered in pulmonary artery endothelial cells after knockdown of apelin (Kim et al., 2013), and miRNA-124, which is more highly expressed in blood outgrowth endothelial cells derived from PAH patients compared to control subjects (Caruso et al., 2017). Since the target prediction of miRNA follows complementary base pair binding, systems approaches may be able to detect broader disease-relevant miRNA independent of vascular cell type. A network based on curated seed genes with known importance in PH and from a master list of functional molecular gene–gene interactions (the ‘consolidated interactome’) has been used to predict miRNAs that potentially interact with PH-related genes (Bertero et al., 2014; Parikh et al., 2012). Systematic screens of miRNA and their combination in patient-derived samples at scale to reveal associations with disease endpoints or endophenotypes are currently lacking, miRNA levels in whole blood may offer a robust opportunity for biomarker development. Measurements of single miRNAs can report clinical outcomes (Rhodes et al., 2013), but a signature comprising a combination of miRNAs is likely to be more informative.

In contrast to snRNAs, which act via RNA binding, IncRNAs exert their regulatory effects at both the transcriptional and post-transcriptional level. IncRNAs can regulate gene expression but are also involved in various other regulatory functions via directly interacting with DNA, such as genomic imprinting and recruiting functional proteins (followed by epigenetic modulation or transcriptional regulation; Yao, Wang, & Chen, 2019). Recent studies have identified IncRNAs that modulate the behaviour of pulmonary artery cells smooth muscle cells and are dysregulated in PAH lung samples (Chen et al., 2018; Jandl et al., 2019; Leisegang et al., 2017). Circular RNAs (circRNAs), the 3' and 5' ends of which are covalently linked, constitute a distinct class of RNA recently discovered to be widespread and abundant in human cells. CircRNAs have been attributed with different functions, including binding snRNA and transcriptional and post-transcriptional regulation of gene expression (Barrett & Salzman, 2016). Despite their abundance in human cells, the functional role of most circRNA remains unknown, and the circRNA transcriptome has not been studied in human pulmonary vascular cells or PAH tissues.

4 | CHANGES IN THE NONCODING TRANSCRIPTOME IN PH

The noncoding transcriptome includes many regulatory transcripts, which can be roughly classified into small noncoding RNAs (sncRNAs, <200 nucleotides), including miRNAs, and long noncoding RNAs (lncRNAs, >200 nucleotides).

5 | PROTEOMIC ADVANCES IN PULMONARY ARTERIAL HYPERTENSION

The measurement of protein levels provides an intermediate phenotype closer to the clinical phenotype of patients than raw
transcriptome data. Strategies for analysing protein levels in tissues differ between the most unbiased in terms of target proteins, such as MS-based methods, and more targeted methods provided, for example, by antibody (e.g. O-link) and aptamer-based (Somalogic) arrays.

An early and unbiased LC-MS study of lung tissue from PAH explants and lobectomy controls identified, among others, elevated levels of the chloride intracellular channel 4 (CLIC4) as a novel pathway involved in PAH (Table 1; Abdul-Salam et al., 2010). Subsequent work has shown that CLIC4 may act through ADP ribosylation factor 6 (Arf6) to regulate endosomal trafficking and modify expression of BMPR2, thus contributing to PAH (Abdul-Salam et al., 2019). Another early proteomic analysis of samples obtained from peripheral muscle has documented down- and up-regulated proteins in PAH compared to healthy subjects. Most of the down-regulated proteins were related to mitochondrial structure and function (Malenfant et al., 2015). A recent proteomic study in pulmonary arterial endothelial cells obtained from PAH patients after lung transplantation found that changes of protein levels in these pulmonary cells can be linked to the mitochondria (Xu et al., 2019).

An analysis of 1,124 plasma proteins from a defined PAH population using the 1K Sommer platform established the potential clinical utility of proteome screening of venous blood; a nine-protein panel was defined that predicts survival at baseline in PAH with an accuracy equivalent to clinical measures, such as provided by the REVEAL prognostic equation (Benza et al., 2019; Rhodes, Wharton, et al., 2017). The proteins cover a range of pathological processes relevant to PAH, such as myocardial stress (IL-1 receptor-like 1 also known as ST-2), inflammatory and metabolism (insulin growth factor binding protein-1 and apolipoprotein E), innate immunity (complement factors H, reduced and D, increased), abnormal iron status (erythropoietin) and thrombogenesis (reduced plasminogen). The prognostic relationship to PAH could be summarized by cut-offs as a simple score from 0 to 9 proteins, which were associated with significantly reduced median survival times in cohorts totalling over 300 patients (Rhodes, Wharton, et al., 2017). Deterioration in the score after initiating targeted therapies was also associated with poorer outcomes compared to patients whose score either stabilized or improved. Interestingly, a similar approach to predict cardiovascular outcomes in coronary heart disease patients also identified a panel of 9 proteins, though the proteins themselves were distinct from the PAH panel (Ganz et al., 2016). Whether a PAH protein panel aids clinical decisions requires testing in prospective clinical studies. It also remains to be established if these proteins represent pathways, which should be targeted therapeutically, or if they simply report on disease progression.

A recent report using a multiplex immunanalysis of 48 cytokines, chemokines and other factors in Group 1 PAH provides support for the concept that molecular profiling might reveal endophenotypes of therapeutic interest (Sweatt et al., 2019). Unsupervised clustering was used to classify patients into proteomic immune clusters without guidance from clinical features. Four clusters were identified that differed in terms of clinical outcomes. Each cluster contained patients with a mixture of PAH aetiologies. The implication of these observations is that the molecular phenotypes could provide a framework for examining drugs that target immunity independent of classical clinical diagnosis. Other proteomic platforms could offer the same opportunity. A larger Somamer panel is now available, which has the advantage of broad coverage, but it also has its limitations (Joshi & Mayr, 2018); independent validation of proteins of interest using an alternative targeted assay is necessary.

6 METABOLIC PROFILING IN PULMONARY ARTERIAL HYPERTENSION

Advances in bio-analytical techniques now permit the quantification of hundreds of metabolites in sample volumes as small as 100 μl, and public databases such as the Human Metabolome Database (www.hmdb.ca) currently comprise information on over 110,000 metabolites (Wishart et al., 2018). Methodologies for measuring metabolites at scale include NMR spectroscopy, MS and HPLC (McGarrah, Crown, Zhang, Shah, & Newgard, 2018; Suhre & Gieger, 2012).

Metabolites offer a functional readout of molecular pathways and are arguably closer to the phenotype than gene expression or protein synthesis. But dissecting the variation in metabolite levels explained by a disease state as opposed to other factors can be challenging. Metabolites are substantially influenced by a wide range of environmental and lifestyle factors, including fasting and feeding states, time of day and menstrual cycle (Suhre & Gieger, 2012). That said, random sampling of plasma metabolites could be illuminating.

Studies of whole lung tissue obtained from lung transplantation in end-stage PAH patients using unbiased/untargeted metabolomics have detected disrupted glycolysis, increased tricarboxylic acid (TCA) cycle, fatty acid metabolites with altered oxidation and decreased arginine metabolism (Zhao et al., 2014; Zhao et al., 2015). Fatty acid metabolism has been addressed specifically in tissue from right ventricular samples obtained at autopsy (Brittain et al., 2016). The adult heart normally obtains 50%-70% of its energy from fatty acid β-oxidation (Sutendra et al., 2010). In heart failure, circulating levels of fatty acids are elevated, but uptake to cardiac tissue may be inhibited. Long chain fatty acids were elevated in plasma and in tissue samples from the right ventricle from PAH patients compared to unaffected controls (Brittain et al., 2016). A potential link between fatty acid metabolism and PAH is BMPR2 dysfunction; in BMPR2 mutant mice, right ventricular tissue is significantly less able to metabolize long chain fatty acids than control tissues (Brittain et al., 2016). Studies targeting a diverse range of lipid species (i.e. lipiddomics; Hinterwirth, Stegemann, & Mayr, 2014) have not been conducted in the context of PAH and are technically challenging, but untargeted metabolomics approaches do cover some lipid categories.

Under physiological conditions, the TCA cycle is fed by several pathways, such as glucose oxidation, β-oxidation and glutaminolysis, to produce NADH/FADH2 required for ATP production in the mitochondrial electron transport chain. The accumulation of TCA intermediates is consistent with dysfunction of the TCA cycle and/or the
electron transport chain downstream, preventing cells from matching their energy demands with energy needs. In a study of dyspneic individuals, the plasma concentrations of 105 pre-selected metabolites were measured and related to pulmonary haemodynamic measures (Lewis et al., 2016). TCA cycle intermediates (aconitate, isocitrate, malate and succinate methylmalonate) were found to associate with the severity of PH in a discovery cohort, and derived metabolite scores were associated with mean pulmonary arterial pressure in a validation cohort (n = 142; Lewis et al., 2016). Inhibition of pyruvate dehydrogenase kinase is a potential therapeutic strategy for improving pyruvate metabolism to fuel the TCA cycle in PAH (Michelakis et al., 2017).

Two recent metabolomics studies have examined plasma lipoproteins in PAH (Harbaum et al., 2019; Hennes et al., 2019). Lipoproteins are closely linked to various vascular homeostatic processes, and increased cardiac and peripheral muscle lipid deposition are found in PAH (Brittain et al., 2016; Malenfant et al., 2015). In a screen of 105 distinct lipoproteins subclasses, higher levels of HDL4, the smallest HDL subclass, were associated with long-term survival (Harbaum et al., 2019). HDLs facilitate lipid efflux from tissues and exhibit vasodilatory, anti-inflammatory and endothelial protective properties. Combining metabolomics and proteomics revealed that the protein cargo of HDL4 particles includes regulators of fibrinolysis, providing a mechanistic explanation for the association, and insight into the conflicting reports of the association of HDL with PAH survival. A study of insulin resistance in PAH showed an elevated triglyceride:HDL ratio, elevated circulating medium- and long-chain acylcarnitines and lipoproteins, and provides evidence that oxidized LDL and its receptor OLR1 may play a role in a pro-inflammatory phenotype in PAH (Hennes et al., 2019).

The largest to-date metabolomics study on plasma samples from PAH patients (n = 365) confirmed previous observations and identified a range of metabolites robustly linked to clinical endpoints in PAH (Table 1; Rhodes, Ghataorhe, et al., 2017). From an untargeted screen of 686 circulating metabolites, a set of 16 was found to discriminate PAH from healthy and disease control groups (symptomatic patients in whom PH was ruled out) and associate with overall mortality in PAH (Rhodes, Ghataorhe, et al., 2017). A central hub metabolite, the modified nucleoside N2,N2-dimethylguanosine, was identified by correlation-based network analysis. Modified nucleosides are dynamically regulated and alterations in expression of tRNAs occur in response to various stimuli, such as cellular stress. The initial cleavage of tRNAs and release into the circulation is mediated by the ribonuclease angiogenin, which was observed to be elevated in the plasma of PAH patients and correlated with levels of modified nucleosides (Rhodes, Ghataorhe, et al., 2017). Both modified nucleosides and angiogenin may reflect increases in both pulmonary vascular cell proliferation and stress in PAH. The most significant shifts in metabolite levels were associated with patients who died during follow-up. Consistent with this, serial samples from patients who died during follow-up were more likely to show a further increase in prognostic metabolites, including circulating modified nucleoside levels, than patients who survived, suggesting that monitoring metabolite levels over time could be useful in judging response to therapy in clinical management of PAH (Rhodes, Ghataorhe, et al., 2017).

7 | OTHER OMICS

Novel methodologies for high-throughput molecular analyses of samples are constantly being refined and developed. Modalities that will provide further characterization of the disease process in PH include breath analysis and microbiome studies. Exhaled NO is already deployed as a clinical biomarker, but more comprehensive screening of volatile organic compounds in breath has potential (Nakhleh, Haick, Humbert, & Cohen-Kaminsky, 2017). A small study of 22 PAH patients and 23 controls harnessing a real-time sensor of unidentified volatile organic compounds distinguished disease from reference samples (Cohen-Kaminsky et al., 2013). More comprehensive studies with larger sample numbers and identification of key molecular pathways are required to fully elucidate the power and biological importance of this approach.

There is a growing awareness that the microbiome has an important impact on our health, the greatest number and variety of bacteria being resident in the gut. Microbiome studies have themselves been assisted by the use of multiple omics approaches to dissect the complex interactions between the gut microbiota, the host and the environment in health and disease (Knight et al., 2018). It is clear that gut microorganisms influence the metabolomic profile of the host, but they also have effects on the immune system (Cani, 2018). For example, cancer patients with higher Akkermansia muciniphila numbers in their gut microbiota demonstrated better responses to immunotherapeutic blockade of programmed cell death protein 1. This was replicated in animal models and driven by increased recruitment of a subset of T lymphocytes to the tumour vasculature following oral A. muciniphila supplementation (Routy et al., 2018). Whether the microbiome of PAH patients provide a further link between the burgeoning evidence of an immune component to its pathobiology remains to be established (Thenappan, Khoruts, Chen, & Weir, 2019).

8 | INTEGRATION OF OMICS TO DEFINE PATHWAYS AND PRIORITIZE CANDIDATES

The integration of multiple omics measurements (e.g. genetic variants, gene expression levels, protein and/or metabolite concentrations) is an active and developing area of research, with evolving applications that systematically investigate the interplay between multiple organizational layers of a biological system (Civelek & Lusis, 2014; Menche et al., 2015; Suhre & Gieger, 2012; Xu et al., 2019). The ‘low hanging fruit’ relies on exploring the relationships between genomic signals and other omics datasets (Suhre & Gieger, 2012). Common genetic variants can influence intermediate phenotypes, such as transcript, protein and metabolite levels, in a quantitative manner (Long et al., 2017; Shin et al., 2014; Suhre et al., 2017; Sun et al., 2018; Westra et al., 2013). Genome-wide mapping of quantitative trait loci
(QTLs) that correlate with these intermediate phenotypes provides a tool to dissect the mode of regulation of gene expression and a functional readout of loci across the genome (Civelek & Lusis, 2014; Suhre & Gieger, 2012). Over-representation of these QTLs in a disease of interest can support the identification of underlying mechanisms by which risk variants mediate disease susceptibility (Yao et al., 2018). The observation that the same genetic variant is driving the association signal in genome-wide association study (GWAS) on a complex disease trait and is affecting expression of, for example, a neighbouring gene, protein abundance or methylation site, allows the inference of causality, a concept captured by Mendelian randomization (MR) studies (Lawlor, Harbord, Sterne, Timpson, & Davey Smith, 2008).

Examples of this strategy are prevalent in common diseases in which genetic variants from GWAS are linked to gene expression QTL (eQTL), protein QTL (pQTL) and metabolite (mQTL) levels (Swerdlow et al., 2012; Yao et al., 2018). Large-scale studies have focused mainly on blood cells as relatively accessible tissues (Long et al., 2017; Shin et al., 2014; Suhre et al., 2017; Sun et al., 2018; Westra et al., 2013). Until sample sizes for eQTL discovery in tissues of interest (particularly heart and lung) grow sufficiently large, blood-based discoveries can serve as proxies (Routy et al., 2018). For eQTLs, recent studies utilizing publicly available GTEx data (https://gtexportal.org/home/) have not found evidence that tissue-relevant eQTLs are enriched for associations with complex traits (Routy et al., 2018). This observation is supported by the latest GTEx release reporting that eQTLs located in cis-regions (i.e., less than 1 Mb of the transcription start site) are much less tissue-specific compared with eQTLs located in trans-regions (more distant from the transcription start site; Figure 3a: Battle, Brown, Engelhardt, & Montgomery, 2017). Trans-acting variants are also more likely to exhibit pleiotropic effects on other transcripts. Directly measured proteins would be expected to more closely report the activity of biological systems, and large-scale pQTL datasets are now also publicly available (Emilsson et al., 2018; Suhre et al., 2017; Sun et al., 2018; Yao et al., 2018).

MR studies have been used successfully to provide credibility for potential therapeutic targets and dissociate on-target from off-target effects (Swerdlow et al., 2012). In PAH, a recent MR analysis has demonstrated the association of iron deficiency with PAH is not causal for disease risk (Ulrich et al., 2020). The instruments used in these studies can also be used to enrich clinical trials with patients more likely to respond. Validated examples are lacking in PAH, but the promise is there (Rhodes, Wharton, et al., 2017). A GWAS on circulating proteins has identified cis-acting pQTLs for proteins, for example, the PDGF β receptor (PDGFRβ), which are known drug targets in PAH (Schermuly et al., 2005). As a potent mitogen and chemoattractant for vascular smooth muscle cells, PDGF is involved in the pulmonary vascular remodelling observed in PAH (Yu et al., 2003). Imatinib is a TK inhibitor that inhibits PDGFR α and β and consistently reverses PH in animal models (Schermuly et al., 2005) and has efficacy in clinical trials (Ghofrani et al., 2010; Hoeper et al., 2013). But there are important safety concerns, so identifying patients most likely to respond would improve the balance of benefit to harm. GWAS studies on circulating levels of PDGFR β (Suhre et al., 2017; Sun et al., 2018) have identified a single pQTL at genome-wide level of significance (Suhre et al., 2017; Sun et al., 2018). This pQTL is located in cis to the PDGFRβ gene. Variants identified with the smallest P value at this locus (e.g. rs2304058, Sun et al., 2018, or rs3776081, Suhre et al., 2017) are co-inherited (linkage disequilibrium with r² = 0.56) and display minor allele frequencies of 45% and 31% in European populations (Karczewski et al., 2019; Machiela & Chanock, 2015). Variation at this locus influences the plasma level of PDGFR β and could influence treatment response to imatinib if PDGFR β was a major mediator of therapeutic effect (Figure 3). The high frequency of variation at this locus in European populations and the cis-acting nature of the variants support its utility in precision medicine. Incorporating this pQTL into a clinical trial could enable intelligent positioning of imatinib as a treatment for PAH.

Increasingly, attention is turning to network approaches which attempts to take account of the large number of potential simultaneous interactions between relevant genes, proteins and metabolites. The current human protein–protein interactome, curated from a variety of sources documenting structure and functional responses, contains over 13,000 proteins and over 140,000 physical interactions

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**FIGURE 3** (a) Cis-acting variants directly influence expression of a target gene while trans-acting variants influence a target gene indirectly via expression of other transcripts, which likely also influence other targets (i.e. horizontal pleiotropy). (b) Integration of proteomic and genomic data allows strategic clinical trial design. For example, cis-acting variants influence circulating levels of PDGF β receptor (PDGFRβ) and could be used to inform the design of a clinical trial of a PDGFRβ inhibitor, such as imatinib, in PAH.
(Menche et al., 2015). It is far from complete and lacks detail around the relative strength, direction and tissue specificity of these interactions (Leopold & Loscalzo, 2018). But this ‘consolidated interactome’ provides a model for examining disease-associated changes and, working within its limitations, has provided some interesting insights into disease endophenotypes and possibilities for drug repurposing (Cheng et al., 2018). Mapping genes, miRNAs and proteins shown to be perturbed in PAH onto the interactome have associated miRNA-21 with a central regulatory role in PH, the role of complement in PAH and novel disease pathways and drug targets (Frid et al., 2019; Parikh et al., 2012; Samokhin et al., 2018). This is just the beginning of an approach with great promise to deliver as more data become available.

9 | PHARMACOGENOMICS

There is a place for pharmacogenomics in PAH. The current empirical approach to treatment selection is expensive (patients may return unused drugs to pharmacy if they do not work or have side effects, but these cannot be re-prescribed to others) and exposes patients to medicines that have side effects but may not improve their PAH. Two examples where the utility of genetic variants in clinical decisions wait to be validated in clinical practice are a transcriptomic signature for responders to calcium antagonists (Hennes et al., 2015) and a variant in the gene GNG2 (rs11157866), a subunit of GPCRs, that may predict treatment response to an endothelin receptor antagonist (Benza et al., 2015). Further efforts to develop pharmacogenomics in PAH, particularly outside formal clinical trials (e.g. using registry data), would benefit from the adoption of internationally accepted criteria defining a therapeutic response to a PAH medicine in routine clinical practice.

10 | CONCLUSIONS

Significant progress has been made in the study of ‘omics profiles’ in PAH in recent years. As costs continue to fall and international collaborations continue to facilitate better-powered studies, the stage is set for this to accelerate. Incorporation of profiling methodologies into standard clinical workups will take time, but genomics is leading the way with benefits to diagnostics already driving national investment programmes. Integration of these datasets will advance our understanding of the pathogenesis of PH and facilitate the definition of patient groups most likely to respond to therapeutic options, and ultimately improve the management of this deadly condition.

10.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018) and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Cidlowski et al., 2019; Alexander, Keely et al., 2019).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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