8ª Reunión de Investigación en Hipertensión Pulmonar

Viernes 1 de Marzo de 2024
10:00 - 17:30

Aula de conferencias. 2ª planta Pabellón 3
Departamento de Farmacología y Toxicología
Facultad de Medicina
Universidad Complutense Madrid

Organización Científica: Línea de Investigación en Hipertensión Pulmonar del CIBERES

Secretaría Técnica: Fundació Privada Món Clínic

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Instituto de Salud Carlos III

Bienvenida

Bienvenidos a la **8ª Reunión de Investigación en Hipertensión Pulmonar**, organizada por la Línea de Hipertensión Pulmonar del Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES).

La reunión tiene por objetivo dar a conocer la investigación que se está realizando actualmente en el campo de la hipertensión pulmonar en España, promover el intercambio de información entre los investigadores y fomentar el desarrollo de acciones colaborativas.

Como en anteriores ediciones, en la presente reunión participan investigadores clínicos de distintas especialidades, investigadores básicos de disciplinas diversas e investigadores traslacionales. La reunión ha sido organizada por el CIBERES, pero no va dirigida sólo a investigadores de dicho centro, sino que está abierta a todos los investigadores que trabajan en el campo de la hipertensión pulmonar en España.

En esta edición se presentarán 21 comunicaciones orales y la conferencia magistral será impartida por el **Prof. Norbert Weissmann, de la Justus-Liebig, Universität Gießen**.

Es nuestro deseo que la reunión sea fructífera y provechosa para todos y que facilite futuras colaboraciones.

Dr. Joan Albert Barberà **Prof. Francisco Pérez-Vizcaíno**

Agenda

Bienvenida **09:30-09:40**

Dra. María Molina-Molina
Directora Científica
CIBERES

Prof. Francisco Pérez-Vizcaíno
Coordinador. Programa de Enfermedades Respiratorias Difusas
CIBERES

Dr. Joan Albert Barberà
Coordinador, Línea de Investigación en Hipertensión Pulmonar
CIBERES

Conferencia Invitada **09:40-10:15**

▪ Tobacco-smoke induced pulmonary hypertension
Prof. Norbert Weissmann
Justus-Liebig, Universität Gießen

Comunicaciones. Sesión 1 **10:15-11:45**

Moderadores:
Jair Tenorio, Olga Tura, Ángel Cogolludo

▪ Novel candidate genes implicated in the development of pulmonary arterial hypertension ▪ 10:15
Lucía Miranda
Instituto de Genética Médica y Molecular (INGEMM)-Hospital Universitario La Paz, Madrid

▪ Unravelling the mechanisms of COPD: role of nAChRs in pulmonary vascular impairment induced by cigarette smoke ▪ 10:30
María José Calzada
Instituto de Investigación Sanitaria Princesa (IIS-Princesa), Madrid

▪ New variants identified in low evidence genes associated with pulmonary arterial hypertension development ▪ 10:45
Natalia Gallego
Instituto de Genética Médica y Molecular (INGEMM)-Hospital Universitario La Paz, Madrid

▪ Effects of physical training on mitochondrial function in a mouse model of pulmonary arterial hypertension ▪ 11:00
Kelly Casós
Hospital Clínic-IDIBAPS, Barcelona

▪ The influence of sex on the progression of pulmonary arterial hypertension in mice ▪ 11:15
María Jesús Sánchez
CIC biomaGUNE-Basque Research and Technology Alliance (BRTA), Donostia

▪ Pulmonary arterial hypertension is induced by altering mitochondrial dynamics in endothelial cells ▪ 11:30
Bertha García-León
Centro de Investigaciones Biológicas Margarita Salas (CIB)-CSIC, Madrid

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Pausa – Café **11:45-12:15**

Comunicaciones. Sesión 2 **12:15-14:00**

Moderadores:
Lucilla Piccari, Víctor Peinado, Jesús Ruiz-Cabello

▪ Study of a novel hypoxia system to evaluate endothelial cell dysfunction in chronic thromboembolic pulmonary hypertension (CTEPH) ▪ 12:15
Olga Tura-Ceide
Hospital Clínic-IDIBAPS, Barcelona

▪ Effects of IL-11 system on human pulmonary artery contractility ▪ 12:30
Javier Milara
Universidad de Valencia

▪ Guanylate cyclase stimulation by Riociguat reverts vascular remodelling through modulation on MAPK in pulmonary artery smooth muscle cells and fibroblasts ▪ 12:45
Adelaida Bosacoma
Hospital Clínic-IDIBAPS, Barcelona

▪ S1R agonist treatment as a novel approach in PAH targeting Kv1.5 channels ▪ 13:00
Marta Villegas-Esguevillas
Universidad Complutense de Madrid

▪ Zinc transporter ZIP12 overexpression in smooth muscle cells induces vascular remodeling and pulmonary arterial hypertension ▪ 13:15
Lola Navarro-Llinares
Centro de Investigaciones Biológicas Margarita Salas (CIB)-CSIC, Madrid

▪ Vitamin D as an add-on therapy to phosphodiesterase-5 inhibitors in experimental pulmonary arterial hypertension ▪ 13:30
Rui Adão
Universidad Complutense de Madrid

▪ Update and management of the Spanish pulmonary hypertension biobank ▪ 13:45
Teresa Botta
Biobanc Hospital Clínic-IDIBAPS, Barcelona

Pausa – Comida **14:00-15:00**

Presentación FCHP **15:00-15:15**

Comunicaciones. Sesión 3 **15:15-17:15**

Moderadores:
Isabel Blanco, Diego Rodríguez, TBD

▪ Assessing the clinical benefit, safety, and patient-reported outcomes with the use of the PAHcare™ digital platform in pulmonary arterial hypertension: a pilot study ▪ 15:15
Carlos Ojeda
Hospital Universitario Doctor Negrín, Las Palmas de Gran Canaria

▪ Gas exchange and hemodynamic impairment in pulmonary hypertension associated with interstitial lung diseases: are they correlated? ▪ 15:30
Lorenzo Volpiano
Hospital del Mar-IMIM, Barcelona

▪ Cardiopulmonary exercise testing with simultaneous echocardiography after pulmonary embolism ▪ 15:45
Karys Khilzi
Hospital del Mar-IMIM, Barcelona

▪ Evaluating PEG coating of antifibrotic nanoparticles for pulmonary delivery — ▪ 16:00
Marina Piñol
CIC biomaGUNE-Basque Research and Technology Alliance (BRTA), Donostia

▪ Nanomedicine for the targeted treatment of venous thromboembolism — ▪ 16:15
Remedios Otero
Virgen del Rocío-CSIC, Universidad de Sevilla

▪ COPD and pulmonary fibrosis associated with severe pulmonary hypertension show different proteomic profiles — ▪ 16:30
Adelaida Bosacoma
Hospital Clínic-IDIBAPS, Barcelona

▪ Towards an omic classification of pulmonary hypertension — ▪ 16:45
Mónica Mora
Instituto de Genética Médica y Molecular (INGEMM)-Hospital Universitario La Paz, Madrid

▪ Dysfunction of endothelial progenitor cells in pediatric pulmonary hypertension: a characterization study — ▪ 17:00
Rebeca Carrión
Universidad Complutense de Madrid

Conclusiones y Despedida — **17:15**

Dr. Joan Albert Barberà
Coordinador, Línea de Investigación en Hipertensión Pulmonar
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Fundación Contra la Hipertensión Pulmonar · Món Clínic Barcelona · MSD · Johnson & Johnson · AOP HEALTH

Comunicaciones. Sesión 1

Novel candidate genes implicated in the development of pulmonary arterial hypertension

Lucía Miranda
Instituto de Genética Médica y Molecular (INGEMM)-Hospital Universitario La Paz, Madrid

Lucía Miranda Alcaraz, Natalia Gallego-Zazo, Alejandro Cruz-Utrilla, María Jesús del Cerro Marín, Manuel López Meseguer, Amparo Moya Bonora, Nuria Ochoa Parra, Alejandro Parra, Patricia Pascual, Mario Cazalla, Cristina Silván, Pedro Arias, Pablo Lapunzina, Pilar Escribano-Subías, and Jair Tenorio-Castano

Pulmonary Arterial Hypertension (PAH) is a rare disease characterized by elevated blood pressure in the pulmonary arteries causing progressive heart failure and leading to premature death. This study aims to select and validate pathogenic variants in genes previously associated with PAH and to investigate new genes and variants potentially involved in the development of the disease.

Fifty-five patients clinically diagnosed with PAH and 21 unaffected family members were analyzed using whole exome sequencing (WES). Analysis of the results was carried out using a bioinformatics algorithm for variant prioritization.

Genetic analysis detected pathogenic variants or VUS (variants of uncertain significance) in 30'9% in genes previously related to PAH, in addition to three VUS in three new candidate genes: ATF2, HDAC5 and UACA. Variants in ATF2 and UACA may influence hyperproliferation and resistance to apoptosis of pulmonary vascular cells and contribute to the development of PAH. In addition, an incorrect functioning of the posttranslational protein acetylation regulatory mechanisms in which the HDAC5 gene participates also contributes to the development of PAH by producing an aberrant epigenetic signature that aggravates the characteristic vascular remodeling process.

Whole exome sequencing allows identification of causal variants in genes previously associated with PAH for diagnosis and allows further study in inconclusive cases to identify variants in new genes potentially implicated in the disease, although further studies are needed to describe the involvement of these genes in PAH.

Unravelling the mechanisms of COPD: role of nAChRs in pulmonary vascular impairment induced by cigarette smoke

María José Calzada
Instituto de Investigación Sanitaria Princesa (IIS-Princesa), Madrid

Rubert-Munar O[1], Andreu RM[1], López-García N[1], Rodríguez-Pérez J[1], Cogolludo A[2,3], and Calzada MJ[1,3]

[1] Departamento de Medicina, Facultad de Medicina, Universidad Autónoma de Madrid. Instituto de Investigación Sanitaria Princesa (IIS-Princesa), Madrid, Spain; [2] Departamento de Farmacología y Toxicología, Facultad de Medicina, Universidad Complutense de Madrid, Madrid, Spain
[3] Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain.

Tobacco smoke is the main environmental risk factor for the development of chronic obstructive pulmonary disease. Our studies focus on the effects of tobacco smoke on chronic obstructive pulmonary disease (COPD). Specifically, we investigated the impact of tobacco smoke extract (CSE) on human pulmonary artery smooth muscle cells (hPASMCs), in particular the effects on ROS production, antioxidant response, calcium signaling, cell structure, and its implications for dysregulation of vascular tone.

Our results demonstrate the involvement of nicotinic channels and the interplay between mitochondrial ROS, Ca2+ dysregulation, cytoskeleton defects and altered vascular tone in the context of COPD and pulmonary hypertension. Furthermore, manipulation of these channels significantly reverses tobacco-mediated effects, underscoring the importance of these pathways as a specific therapeutic strategy for the treatment of PH secondary to COPD.

New variants identified in low evidence genes associated with pulmonary arterial hypertension development

Natalia Gallego
Instituto de Genética Médica y Molecular (INGEMM)-Hospital Universitario La Paz, Madrid

Natalia Gallego-Zazo[1,2,3], Lucía Miranda-Alcaraz[1,2,3], Mónica Mora[1,2,3], Alejandro Cruz-Utrilla[4,5], María Jesús del Cerro Marín[6], Manuel López Meseguer[7], Amparo Moya Bonora[8], Nuria Ochoa Parra[4,5], Alejandro Parra[1,2,3], Patricia Pascual[1,2,3], Mario Cazalla[1,2,3], Cristina Silván[1,2,3], Pedro Arias[1,2,3], Julián Nevado[1,2,3], Pablo Lapunzina[1,2,3], Pilar Escribano-Subías[4,5], and Jair Tenorio-Castano[1,2,3]

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Pulmonary arterial hypertension (PAH) is a rare vasculopathy with significant morbidity and mortality. Genetic testing is currently recommended for adults and children diagnosed with PAH. Variants in at least 27 genes have putative evidence for PAH causality. An international panel of experts in PAH applied a scoring system to classify the relative strength of evidence supporting PAH gene-disease relationships based on genetic and experimental evidence. According to this classification, twelve genes were classified as having definitive evidence, three with moderate, six were classified as having limited evidence, five genes were disputed because of a paucity of genetic evidence over time and one was classified as having no known PAH relationship. Therefore, there are several genes that require further study to definitively associate them with PAH.

We performed a retrospective study on 530 patients from the Spanish registries of adult and pediatric patients with PAH (REHAP and REHIPED). In all of them, whole exome sequencing was performed and a variant prioritization algorithm was designed to prioritize variants present in the 14 genes with moderate, limited and disrupted evidence of association with PAH.

We have found 60 variants of unknown significance in the 14 studied genes, i.e., the 11% of the patients showed variants in these genes. It should be noted the variants detected in three of these genes: *NOTCH1* (15 variants), *ABCC8* (12 variants) and *NOTCH3* (11 variants).

We add genetic evidence to the relationship of some genes associated with PAH, specially, *NOTCH1*, *NOTCH3* and *ABCC8*. It would be recommended that genetic testing includes genes with moderate, limited and disrupted evidence although caution must be taken in the interpretation of the variants identified in this genes. It would be necessary more studies in higher cohorts and functional assays to improve our knowledge about the association of this variants with the disease.

Effects of physical training on mitochondrial function in a mouse model of pulmonary arterial hypertension

Kelly Casós
Hospital Clínic-IDIBAPS, Barcelona

Kelly Casós[1], Irene Gómez[1], Olga Tura-Ceide[1,2,3], Joan A. Barberà[1,2], Isabel Blanco[1,2], Víctor I Peinado[2,4]

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Pulmonary arterial hypertension (PAH) is a progressive disease characterized by elevated pulmonary vascular resistance leading to right heart failure. Exercise training has demonstrated improvements in PAH patients' quality of life, exercise capacity, lower limb muscle strength, and possibly hemodynamics.

The study hypothesized that exercise's benefits in the lungs might involve a partial inhibition of the electron transport chain (ETC) complexes (CI–CV), potentially leading to a shift in the phosphorylative phenotype of ATP production resulting in lower lipid peroxidation. Experiments were conducted on a mouse model of PAH exposed to SU5416+hypoxia, with half subjected to a daily moderate exercise program.

The study included four groups: two control groups (exercise (CE) n=6; and sedentary (CS) n=6) and two PAH groups (exercise (HE) n=6 and sedentary (HS) n=6). ETC complexes were examined in lung, heart, and skeletal muscle homogenates through western blot analysis.

In the lungs, the CE vs. HE groups showed a decrease in CIII expression (p=0.038) and in the HS vs. HE groups (p=0.034). There was also a decrease in CV expression comparing CE and HE groups (p=0.045). In the heart, all ETC complexes showed a decrease in the CE vs. HE groups (CI: p=0.015; CII: p=0.02; CIII: p=0.012; CIV: p=0.006; CV: p=0.034) and HS vs. HE groups (CIII: p=0.045). In skeletal muscle, there was a trend towards increased CV levels in the HE groups, reaching levels similar to the CS group.

The findings suggest that exercise may partially inhibit the ETC in the lungs and the heart but not in the skeletal muscle, potentially benefiting PAH and aligning energy production. Further research is needed to fully understand exercise's impact on mitochondrial function in PAH.

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The influence of sex on the progression of pulmonary arterial hypertension in mice

María Jesús Sánchez
CIC biomaGUNE-Basque Research and Technology Alliance (BRTA), Donostia

María Jesús Sánchez-Guisado[1,2], Irati Aiestaran-Zelaia[1,2,3], Marta-Beraza[1,2], Ian J. Holt[3], Jesús Ruiz-Cabello[1,2]

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Introduction: Pulmonary arterial hypertension (PAH) is a rare disease characterized by pulmonary artery remodeling and right ventricle (RV) failure. While the incidence is higher in women, men present a more unfavorable prognosis, and the reasons for sex differences in PAH pathogenesis remain unclear. Identifying these mechanisms is pivotal for tailoring treatment approaches to both sexes. This study aimed to explore the impact of sex on PAH development in mice in vivo.

Methods: PAH was induced in 8-week-old female and male C57BL/6J mice by using the Hypoxia/SU5416 Model. Mice were functionally characterized by ^{18}F-FDG PET/CT imaging for measuring glucose uptake and cardiac MRI for heart function characterization. After that, Mice were euthanized, and heart tissue underwent proteomic and immunofluorescence analysis.

Results/Discussion: The role of RV function is crucial in predicting the prognosis of PAH patients. Conventionally, it is believed that female PAH individuals have better RV function than males. However, our study challenges this notion, revealing that female PAH mice exhibit more severe RV dysfunction, particularly systolic dysfunction, interventricular septum flattening, and LV dysfunction (Figure 1). Interestingly, RV dysfunction in both genders correlates with increased glucose uptake and cell hypertrophy. Yet, females also stand out with heightened LV glucose uptake, strongly correlated with LV atrophy and cardiomyocyte hyperplasia. Our proteomic data from the LV of PAH female mice strongly suggest the presence of mitochondrial dysfunction, a reduction in fatty acid β-oxidation, and an increase in glycolysis in this ventricle, which collectively support the metabolic changes observed by PET.

Conclusion: In summary, our study reshapes the PAH landscape by questioning traditional views on gender-related disparities in RV function. These results underscore the complex nature of PAH, highlighting sex-specific and ventricle-specific nuances crucial for disease progression. Moving forward, essential research is needed to uncover underlying mechanisms, enabling the translation of these insights into targeted therapeutic strategies.

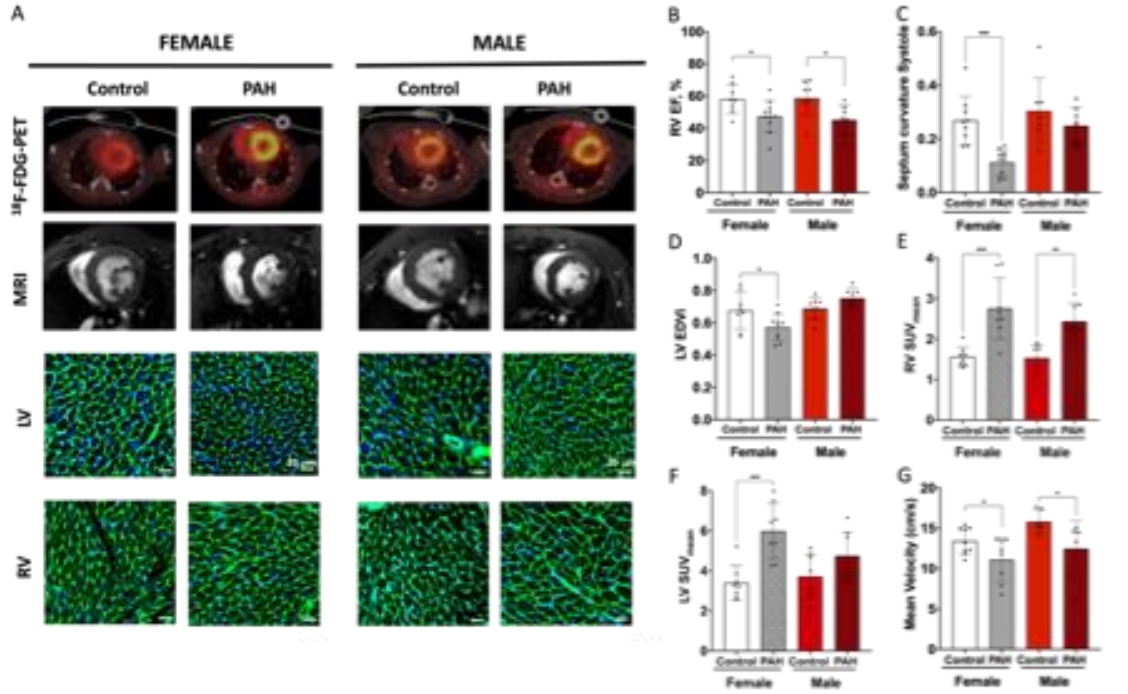

Figure 1. Sex-differences in the progression of pulmonary arterial hypertension. (A) Representative images from the top to bottom of: representative mid-ventricular axial ^{18}F-FDG PET/CT; Short-axis magnetic resonance images of the middle ventricle of control and PAH mice during diastole; images of WGA (green) staining in LV and RV tissues; (B) RV Ejection fraction (EF); (C) Septum curvature systole; (D) LV End-diastolic index; Quantitative analysis of relative RV (E) and LV (F) ^{18}F-FDG PET/CT; (G) Mean velocity (cm/s) at the main trunk of pulmonary artery. Data are presented as the mean ± SD. *p-value < 0.05 (Control vs PAH), assessed using an unpaired t-test.

Pulmonary arterial hypertension is induced by altering mitochondrial dynamics in endothelial cells

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Pulmonary Arterial Hypertension (PAH) is a rare disease characterized by an early development of endothelial dysfunction and pulmonary vascular remodeling caused by aberrant smooth muscle cell proliferation and endothelial-mesenchymal transition. Subsequently, an elevated pulmonary artery pressure and right ventricle hypertrophy is induced causing heart failure and premature death. Interestingly, mitochondrial dysfunction has been related to PAH.

Previously, we described the ATP-dependent metalloprotease YME1L as an essential player in the balance of mitochondrial dynamics and its specific lack within the cardiomyocyte has been reported to induce heart failure (Wai et al., 2015). Now, we hypothesize that the specific lack of *Yme1l* in the endothelial cell will alter mitochondrial dynamics thus disrupting cell homeostasis which will cause endothelial dysfunction and PAH. To answer this hypothesis, we developed an endothelial *Yme1l* knockout (eYKO) murine model and studied its vascular health during aging. For the characterization of eYKO we used various techniques of molecular biology, histology and functional assays to elucidate whether or not eYKO mice developed PAH. Our results demonstrate that eYKO mice presented higher right ventricular systolic pressure and right ventricle hypertrophy along with vascular remodeling and endothelial dysfunction starting from a young age and worsening upon aging. Moreover, the exposure of eYKO to hypoxia exacerbates the PAH phenotype, highlighting the relevance of mitochondrial homeostasis in the development and evolution of PAH. In conclusion, we described a new animal model of PAH induced by altered mitochondrial dynamics in the endothelial cell that recapitulates some of the main pathophysiological characteristics of PAH which worsens upon aging. Furthermore, eYKO mice stands as a dual-hit model to study the impact of preexisting endothelial damage in the onset and evolution of pulmonary vascular diseases.

Our results open new avenues for deeply understanding the disease and finding novel therapeutic approaches to treat PAH

Comunicaciones. Sesión 2

Study of a novel hypoxia system to evaluate endothelial cell dysfunction in chronic thromboembolic pulmonary hypertension (CTEPH)

Olga Tura-Ceide
Hospital Clínic-IDIBAPS, Barcelona

Ylenia Roger, Isaac Almendros, Esther Marhuenda, Adelaida Bosacoma, Anna Sardiné, Irene Gómez, Andrés Urrutia, Ana Ramírez, Victor I. Peinado, Isabel Blanco, Manuel Castellà, Joan A. Barberà, Olga Tura-Ceide

Rationale: Oxygen (O_2) plays a key role in respiratory diseases and hypoxia can be essential in the progression of diseases such as CTEPH. The endurance of hypoxic conditions can contribute to a metabolic shift characterized by an abnormal cell proliferation, tissue hypertrophy and remodelling.

The aim of the study is to evaluate the effect of chronic hypoxia on endothelial cells (ECs) derived from patients with CTEPH (EC-CTEPH) compared to healthy controls and to compare physical and chemical hypoxia.

Methods: Both patient and control cells were cultured under different O_2 conditions (1% and 21% O_2) for 48h. Chemical hypoxia was induced with 500µM DMOG for 24h in both patient and control cells. qRT-PCR for angiogenic and metabolic genes and supernatant (SN) analysis were performed using EC-CTEPH (n=6) and EC-Control (n=6).

Results: Both cell populations exhibit an upregulation of hypoxic responsive genes, such as Vegf or Fih, in response to hypoxia. Notably, genes associated with the glycolytic pathway, such as Hk2 or Ldha, show upregulation only in control cells exposed to 1% O2, with no significant upregulation observed in EC-CTEPH. This observation aligns with an absence of lactate production in EC-CTEPH.

Interestingly, when hypoxia is induced chemically, mRNA relative expression in EC-CTEPH is normalized and becomes comparable to EC-Control. This normalization suggests that the metabolic reprogramming observed in EC-CTEPH under natural hypoxia does not occur when hypoxia is induced chemically.

Conclusions: Metabolic reprogramming seen in EC-CTEPH under hypoxia does not happen when hypoxia is induced chemically. This suggests that glycolytic impairment observed in EC-CTEPH may not be due to Hif-1 signalling pathway. Further investigation is warranted to elucidate the specific mechanisms underlying this unique metabolic behavior in EC-CTEPH.

Funding and acknowledgements. SOCAP, SEPAR, ISCIII (CP17/00114, PI18/00960), CIBERES, FCHP, CPII22/00006, PI21/01212

Effects of IL-11 system on human pulmonary artery contractility

Javier Milara
Universidad de Valencia

Javier Milara, Ines Roger, Paula Montero, Julio Cortijo

Introduction: Group I of pulmonary arterial hypertension (PAH) is a rare disease with a very poor prognosis. Currently, there is no known cure for PAH, except for transplantation. Recent findings provided by our group indicate a role for IL-11 system on pulmonary artery remodeling in *in vivo* and *in vitro* systems, showing an elevation of right ventricle systolic pressure, pulmonary arterial and right ventricle remodeling *in vivo* as well as human pulmonary arterial smooth muscle proliferation and endothelial to mesenchymal transition *in vitro*. However, there is no evidence on the role of IL-11 system on pulmonary arterial contractility.

Objectives: To study the effects of IL-11 system on pulmonary arterial contractility.

Methods and Results: 3D human precision cut lung slice (hPCLS) were obtained from healthy lung explants from the transplant program of the General University Hospital of Valencia, Spain. hPCLS were mounted on a perfusion chamber and visualized in a 40x inverted microscope with a video-camera recording one image each 10 seconds. Perfusion of growing concentration of IL-11 and soluble IL-11Rα (0.1nM-10μM) did not modify basal pulmonary arterial area, showing no direct effect on contractility. In other experimental protocol, hPCLS were stimulated with IL-11 or IL-11Rα during 120h in cell culture conditions, followed by the stimulation with endothelin 1 (0.01-1μM). The IL-11/IL-11Rα pre-treatment increased the potency and maximum effect of endothelin compared with untreated hPCLS. The IL-11/IL-11Rα pre-treatment increased the expression of endothelin A and B receptors and pulmonary artery remodeling markers ACTA2 and TWIST-1, and reduced the endothelial markers CD31, eNOS and FVIII in pulmonary arteries of hPCLS. Human pulmonary arterial smooth muscle cells pretreated with IL-11/IL-11Rα increased the effects of endothelin 1 on intracellular calcium release, explaining the indirect effects of IL-11 system on pulmonary arterial contraction.

Conclusions: IL-11 system participated in pulmonary arterial contraction induced by endothelin 1

Guanylate cyclase stimulation by Riociguat reverts vascular remodelling through modulation on MAPK in pulmonary artery smooth muscle cells and fibroblasts

Adelaida Bosacoma
Hospital Clínic-IDIBAPS, Barcelona

Adelaida Bosacoma, Irene Gómez-Hernández, Anna Sardiné, Ylenia Roger, Daniel Aguilar, Kelly Casós, Isabel Blanco, Olga Tura-Ceide, Joan A. Barberà and Victor I. Peinado

Guinea pigs chronically exposed to cigarette smoke (CS) showed processes of apoptosis and pulmonary vascular remodelling. Administration of a stimulator of soluble guanylate cyclase (sGC) to increase cGMP levels not only had the effect of pulmonary artery relaxation, but also had anti-remodelling effects for mechanisms still unknown. The aim of the present study was to evaluate *in vitro* the effects of sGC stimulation by Riociguat on MAPK pathways in primary pulmonary cells exposed to CS extract (CSE).

Human pulmonary artery endothelial cells (HPAEC), smooth muscle cells (PASMC) and fibroblasts (NHLF) were exposed to CSE (dilution 1/5) or to the combination of CSE and Riociguat (100uM). We analysed proliferation, gene expression of MAPK pathways with a Human TaqMan™ Array-plate and the expression of apoptotic and proliferative genes with qRT-PCR.

CSE induced a dose-dependent proapoptotic effect in the three cell types (p-values<0.05). Besides, Riociguat reverted CSE effect in PASMC (p-value<0.05) but in NHLF it induced apoptosis (p-value<0.05) and in HPAEC had no effects. The study on MAPK pathways revealed that CSE reduced p38 pathway's gene expression and increased phosphatases in PASMC (p-values<0.05). Also in PASMC, Riociguat decreased expression of phosphatases and of MAP3K in the three pathways ERK, JNK and p38 (p-values<0.05). On the contrary, in NHLF, CSE had no significant effects in none of the three MAPK pathways, but Riociguat increased phosphatases' expression and expression of genes in ERK and JNK (p-values<0.05).

These results show that sGC stimulation have antagonistic effects on MAPK pathways depending on the cell type. This modulation in PASMC and fibroblasts could explain the vascular anti-remodelling effect of sGC stimulation.

Supported by FIS PI16/01147, SEPAR 888/2019 and SEPAR 1240/2021.

S1R agonist treatment as a novel approach in PAH targeting Kv1.5 channels

Marta Villegas-Esguevillas
Universidad Complutense de Madrid

Marta Villegas-Esguevillas, Rui Adão, Daniel Morales-Cano, Bianca Barreira, Miguel Olivencia, María Sancho, Laura Moreno, Belén Climent, Francisco Pérez-Vizcaíno,Angel Cogolludo

Kv1.5 channels are key players in the regulation of vascular tone and their impairment is associated with cardiovascular diseases, including pulmonary arterial hypertension (PAH). Unfortunately, pharmacological strategies to improve Kv1.5 channel function are lacking. Recent studies from our lab have shown that sigma-1 chaperone receptor (S1R) agonists arecapable of positively regulating these channels and represent a new strategy to improve their function.

We found that the S1R agonist PRE084 increased the expression of the channel and markedly increased the activity of Kv1.5 currents in pulmonary artery smooth muscle cells which attenuated vasoconstriction and proliferation in the pulmonary arteries. And of note, PRE084 increased the function of Kv1.5 under conditions of sustained hypoxia, as an *in vitro* PAH model. Therefore, our objective in the present study was to evaluate the pharmacological effects of Pre084 in an *in vivo* model of PAH. Differents hemodynamic, electrophysiological and vascular reactivity study techniques were used in pulmonary arteries (PA) obtained from control rats (maintained in normoxia), PA from the PAH animalsmodel (induced by hypoxia + SUGEN) and from animals from the PAH model treated with PRE084. We observed that PRE084 significantly increased Kv1.5 currents in pulmonary artery smooth muscle cells, leading to improved endothelial function and decreased vasoconstriction in response to serotonin. Furthermore, it decreased the Fulton index and reduced the systolic pressure of the right ventricle.

Taken together, our study provides insights into PRE084, demonstrating its capacity to enhance vasodilatory and hemodynamic function in an *in vivo* PAH model, thereby identifying this novel S1R agonist as a potential new drug for the treatment of this disease.

Zinc transporter ZIP12 overexpression in smooth muscle cells induces vascular remodeling and pulmonary arterial hypertension

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Pulmonary vascular remodeling is a hallmark of Pulmonary Arterial Hypertension (PAH). The aberrant proliferation of vascular cells is still one of the targets to beat when fighting this devastating disease. We previously described the zinc transporter ZIP12 as a key regulator of the pulmonary vascular response to chronic hypoxia (Zhao, Oliver et al, 2015). More in detail, we described how its expression was upregulated by HIF1/2alpha in hypoxic conditions resulting in higher intracellular zinc uptake. This change, induced pulmonary vascular remodeling followed by a raise in pulmonary arterial pressure and right ventricular hypertrophy. ZIP12 emerged as a potential therapeutic target, however the exact molecular mechanism and its role in different vascular cell types remains unknown.

In order to further explore the role of ZIP12 in the genesis of PAH, we have worked with human pulmonary arterial smooth muscle cells (HPASMCs) and generated two animal models: the global ZIP12-KO and the conditional R26/ZIP12/tmt-TG transgenic mice. The backcrossing of the last with a SM22-Cre line resulted in the specific human ZIP12 overexpression in smooth muscle.

Our results demonstrate that genetically induced overexpression of ZIP12 in HPASMC was enough to increase intracellular zinc uptake, cell proliferation and migration in vitro. Additionally, mice over-expressing human ZIP12 in SMC presented vascular remodeling and higher right ventricular systolic pressure. Moreover, the exposure of ZIP12-KO mice to hypoxia resulted in an abrogated PAH phenotype, confirming the relevance of this zinc transporter in the development of the disease. The role of ZIP12 in endothelium and its cross-talk with SMC remains to be explored.

In summary, we found that ZIP12 overexpression in SMCs was enough to induce a mild PAH phenotype. More importantly, we have described a new transgenic model of PAH induced by human ZIP12 overexpression that can be used as a tool for testing novel therapeutic interventions.

Vitamin D as an add-on therapy to phosphodiesterase-5 inhibitors in experimental pulmonary arterial hypertension

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Vitamin D (vitD) deficiency is highly prevalent in patients with pulmonary arterial hypertension (PAH). Moreover, PAH-patients with lower levels of vitD have worse prognosis and, in animalswith PAH and deficit of vitD, restoring vitD levels attenuates PAH. Also, recent evidence suggests that vitD deficiency may cause insufficient response to phosphodiesterase-5 inhibitors (PDE5i) in some PAH patients. In this study, we hypothesize that the recovery of optimal vitD levels in experimental PAH might help to improve responsiveness to PDE5i therapy.

Thus, we fed male Wistar rats with a vitD-free diet for five weeks and then received a single dose of Su5416 (20mg/Kg) and were exposed to vitD-free diet and chronic hypoxia (10%O2) for three weeks. Next, vitD deficient rats with PAH were housed in room air and divided into two groups: (a) tadalafil therapy (daily;oral;10mg/kg) + vitD-free diet or (b) tadalafil therapy + single oral dose of 50,000 IU/Kg of vitD plus standard diet for four weeks. Rats were then usedfor cardiovascular functional evaluation.

Recovering optimal levels of vitD improved pulmonary endothelial function, measured by a higher endothelium-dependent vasodilation to acetylcholine, and increased the vasodilator response to sildenafil. Also, pulmonary small artery remodeling was decreased in vitD-restoredgroup. In morphometric analysis, vitD treatment attenuated increases in both right ventricle (RV) and right atrial hypertrophy, as well as the fulton index. VitD supplementation improved the exercise capacity during the endurance tests. Consistently, in the vitD-restored group, RVcatheterization revealed a lower systolic RV pressure, and serial echocardiographic analysis demonstrated an improved pulmonary flow and RV contractility.

In conclusion, the recovery of vitD status might help to improve responsiveness to PDE5i. Besides to comorbidities, detecting clinical traits, as vitD deficiency could allow physicians to individualize the treatment approach.

Update and management of the Spanish pulmonary hypertension biobank

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The Spanish Pulmonary Hypertension bank (BEHIP) was established in 2013 with the aim to collect a well-characterized repository of biological samples from patients with pulmonary arterial hypertension (PAH) or chronic thromboembolic pulmonary hypertension (CTEPH) to promote and facilitate biomedical research. Clinical information is available at the Spanish Pulmonary Arterial Hypertension Registry (REHAP). In addition, samples from pulmonary hypertension associated with respiratory diseases have been incorporated to the repository, with linked clinical information available at the Spanish Registry of Pulmonary Hypertension Associated with Respiratory Diseases (REHAR). In this communication we provide an update of the BEHIP functioning and outcomes focusing on the collection of samples and on the objective of increasing sample procurement to research.

Donors come from centers participating in the REHAP or REHAR registries. Blood samples are collected to obtain back-up total blood, DNA, plasma, serum, and peripheral blood mononuclear cells (PBMCs). Fresh blood samples are processed and stored at the Biobank HCB-IDIBAPS, as well as coded and linked to the clinical data registries for subsequent traceability and clinical characterization.

The BEHIP has collected samples from 717 donors obtained at 8 Spanish hospitals with 12 different diagnoses: idiopathic PAH (n=163), hereditary PAH (n=14), drug-induced/toxic PAH (n=11), Scleroderma (n=56), PAH associated with connective tissue diseases (n=51), PAH associated with HIV infection (n=42), PAH associated with portal hypertension (n=31), PAH associated with congenital heart disease (n=48), multifactorial PAH (n=12), CTEPH (n=238), pulmonary hypertension associated with respiratory diseases (n=38), and other forms of PAH (n=13). The generated aliquots are 678 extracted DNA, 1705 normalized DNA, 481 back-up total blood, 6336 plasma, 5097 serum and 1299 PBMCs aliquots. In addition, samples are linked to their clinical information contained in national registries. Noteworthy, until the end of 2023, 552 aliquots have been procured (353 plasma, 123 DNA, 76 serum), which includes 37.8% of total donors (271/717).

BEHIP is the result of a well-established synergy of multidisciplinary collaborations between clinical/research centers and HCB-IDIBAPS Biobank platform and has a high strategic value. Finally, sample procurement to researchers should be promoted and enhanced to foster biomedical research.

The authors are deeply in debt to the sample donors and their relatives, as well as to the technical staff from Biobank HCB-IDIBAPS and professionals from the clinical centers. Founded by: ISCIII (PI12/00510), SEPAR, CIBERES and FCHP.

Comunicaciones. Sesión 3

Assessing the clinical benefit, safety, and patient-reported outcomes with the use of the PAHcare™ digital platform in pulmonary arterial hypertension: a pilot study

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La HAP es un trastorno circulatorio que requiere de un cuidadoso seguimiento. No existen estudios sobre la aplicación de productos médicos digitales para el autocuidado. PAHcare es una plataforma digital de apoyo con contenido educativo, que recopilar y almacenar información de salud.

Métodos: Estudio prospectivo, no controlado, multicéntrico, con el objetivo principal de evaluar PAHcare en la calidad de vida relacionada con la salud (CVRS). Los objetivos secundarios fueron otros parámetros clínicos, así como el grado de participación y la satisfacción de los pacientes. La plataforma fue evaluada en 53 pacientes, (5 centros) durante 6 meses.

Resultados: Se observaron cambios mínimos, no significativos, en la CVRS y otros parámetros clínicos. El primer mes, el 92% de los pacientes utilizaron la aplicación, y realizaron un total de 2.912 interacciones. Al final de los 6 meses, el 70 % aún la usaban. El 45 % de los pacientes realizaron el primer nivel de formación estructurada. En la primera semana, el 76 % contactó con su asesor de salud, y al final del estudio 28 % mantenía el contacto. De promedio, realizaron casi 8 contactos por paciente. El 92 % de los pacientes se mostraron bastante (29 %) o muy satisfechos con la plataforma (63 %), el 68 % valoró excelente la calidad del servicio, el 74 % aseguró volver a usar la plataforma, el 84 % consideró que se habían resuelto sus necesidades (47 % totalmente, 37 % en gran parte) y el 95 % declaró que recomendaría su uso a un amigo que tuviera HAP. Cinco pacientes (11 %) tuvieron incidentes con PAHcare, ninguno grave.

Conclusiones: Los pacientes con HAP mostraron un uso elevado de PAHcare y gran satisfacción con el servicio prestado para ayudarles a hacer más fácil el control de la enfermedad.

Gas exchange and hemodynamic impairment in pulmonary hypertension associated with interstitial lung diseases: are they correlated?

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Background: Pulmonary Hypertension (PH) frequently complicates interstitial lung disease (ILD), worsening prognosis and quality of life.

Methods: The REHAR (Registro Español de Hipertensión Pulmonar Asociada a Enfermedad Respiratoria) is a multicentric registry collecting data from patients with right heart catheterization (RHC)-determined PH with respiratory diseases. Variables collected include RHC, systolic pulmonary arterial pressure (PAPs) and tricuspid annular plane systolic excursion (TAPSE) on echocardiography, pulmonary functional tests (PFTs) and arterial blood gases (ABG).

Population: We analysed data from PH-ILD patients, correlating PFTs data with haemodynamic parameters at the time of diagnosis.

Results: The group (n = 209) was comprised of prevalently male former smokers 65 years old on average and intermediate FC (II-III). Underlying diagnosis were idiopathic pulmonary fibrosis (44%), nonspecific interstitial pneumonia (14%) and connective tissues disease (12%). They had a restrictive pattern with severely decreased DL_{CO}, normocapnic hypoxia on room air and reduced 6-minutes walking distance with severe desaturation. PH was severe in 41% according to pulmonary vascular resistance (PVR), with moderate decrease of cardiac index (CI). We found a statistical correlation between arterial pressure of carbon dioxide ($PaCO_2$) and echocardiographic and hemodynamic parameters ($PaCO_2$-TAPSE/PAPs ratio r =0.195, p =0.045; $PaCO_2$–PVR r=0.169, p =0.014; $PaCO_2$–CI r =0.162, p =0.026).

Conclusions: $PaCO_2$ is correlated with hemodynamic severity in a large cohort of patients with PH associated with ILD. These findings suggest a simple, non-invasive parameter which, in conjunction with others, could be useful to evaluate the progression of PH. Further studies are needed to evaluate the potential role of $PaCO_2$ in follow-up of PH-ILD patients.

Cardiopulmonary exercise testing with simultaneous echocardiography after pulmonary embolism

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Chronic thromboembolic pulmonary hypertension (CTEPH) represents a late serious complication of acute pulmonary embolism (PE). Early detection of the disease is a pivotal factor to implement an appropriate treatment. Although current guidelines recommend standard cardiopulmonary exercise testing (CPET) to evaluate symptomatic patients after PE, CPET with simultaneous echocardiography could provide relevant information to evaluate right ventricular-pulmonary arterial coupling and identify patients at risk to develop CTEPH. The aim of this retrospective study was to investigate exercise-induced changes in echocardiographic variables of RV function or RV- arterial coupling in patients with residual thrombotic defects at three months after PE.

This retrospective study investigated patients with residual thromboembolic disease on V/Q scintigraphy, persistent symptoms despite adequate anticoagulation after 3 months of acute PE and rest echocardiography without any data that suggest PH. At rest and during exercise, CPET and doppler echocardiography was performed following a standard protocol. Patients were followed over a 2-year period after the PE episode.

Forty-five patients were included completing a follow-up period of at least 24 months. The mean age was 63 (15) years, and 24 (53%) patients were male. Four patients developed CTEPH after two years follow up. Correlation analyses showed that the peak TAPSE was significantly associated with peak workload ($r = 0.454$, $p = 0.003$), peak VO2 ($r = 0.558$, $p < 0.001$), VE/VECO2 (AT) ($r = - 0.531$, $p < 0.001$), and oxygen pulse ($r = 0.375$, $p = 0.02$). The percentage of change on TAPSE/PASP (from rest to peak) was significantly different between patients with or without CTEPH after two years follow up

CPET with synchronic echocardiography could be a useful tool in early assessment of symptomatic patients with perfusion defects on imaging after three months of correctly treated PE without suggestive data of PH at rest.

Evaluating PEG coating of antifibrotic nanoparticles for pulmonary delivery

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Drug encapsulation within nanocarriers and local administration to the lungs have the potential to mitigate the side effects associated with existing treatments for pulmonary fibrosis (PF) as pirfenidone (PFD). Pulmonary delivery faces challenges due to the different lung defenses against inhaled pathogens and particles. In this context, the role of polyethylene glycol (PEG) in pulmonary administration is controversial. While PEG is recognized for its antifouling and enhanced mucus penetration, it can trigger adverse immune responses when conjugated to different molecules and nanocarriers.

This study aims to investigate the use of lipid nanoparticles with PEG (LP-PEG) or without PEG (LP) to examine nano-bio interactions and determine the most efficient nanocarrier for lung delivery, ultimately improving the therapeutic effect of PFD. We made the two liposomes with DPPC the main component of the lung surfactant. Our results indicate that PEG enhances mucus penetration but has no significant effect on lung surfactant corona formation or drug release after incubation with native lung surfactant. Moreover, in vitro experiments with human lung myofibroblasts (HLF) demonstrate that PEG reduces cellular uptake however, both liposomes inhibit fibroblast differentiation into myofibroblasts, as well as cell migration and proliferation. Biodistribution studies in mice with MRI and histology reveal that both types of liposomes can reach deep lung regions and remain there for up to 6 days favoring sustained drug delivery. Finally, we evaluated their ability to potentiate the PFD therapeutic effect in vivo with the bleomycin mouse model of PF. The results of computed tomography (CT), histology and bronchoalveolar lavage analysis show that both liposomes attenuate fibrosis progression by reducing collagen deposition and inflammation.

In conclusion encapsulation of PFD in liposomes improves its therapeutic effectiveness in vitro and in vivo, however, PEG does not influence this improvement.

Nanomedicine for the targeted treatment of venous thromboembolism

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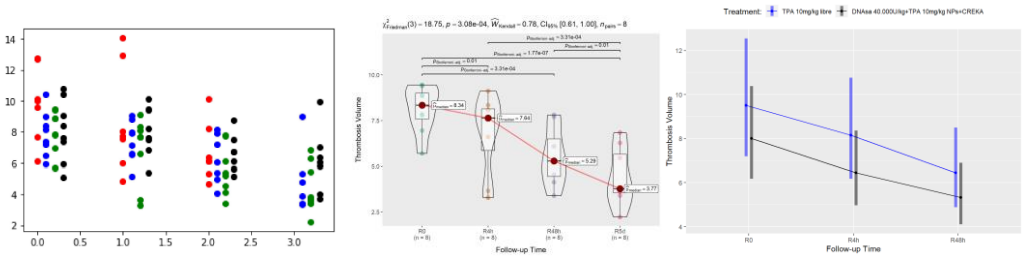
Venous thromboembolism (VTE) is a clinical condition that includes two differentiated pathologies, deep vein thrombosis (DVT) and pulmonary embolism (PE), unresolved PE may leave chronic thromboembolic pulmonary hypertension (CTEPH). Administration of fibrinolytic agents is necessary in some cases of VTE despite the high risk of hemorrhagic complications. A nanomedicine has been designed and developed for the treatment of VTE. Cysteine–arginine–glutamic acid–lysine–alanine clot-binding peptide (CREKA) was conjugated to nanoparticles to release two drugs: *tissue-type plasminogen activator* (tPA) and *recombinant human DNase*.

Our objectives were to evaluate the effects of these nanoparticles in a model of vena cava thrombosis in C57BL6/J mice by i.v. administration. Thrombus volumes were obtained on magnetic resonance imaging at different follow-up stages.

Our study showed the results of different nanoparticles according to the drugs administered (figure A), follow-up thrombosis volume when treated with DNAsa 40.000U/kg +TPA 10mg/kg targeting thrombus with CREKA nanoparticles (figure B) and a generalised linear mixed model (C) of DNAsa 40.000U/kg +TPA 10mg/kg targeting thrombus with CREKA nanoparticles and usual fibrinolytic treatment (TPA 10mg/kg).

A) B) C)



Conclusions: Combined thrombus-targeted by CREKA nanoparticles delivering DNAsa 40.000U/kg +TPA 10mg/kg may be slightly better at reducing thrombus than usual fibrinolytic therapy and potentially safer.

COPD and pulmonary fibrosis associated with severe pulmonary hypertension show different proteomic profiles

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Pulmonary hypertension (PH) associated with chronic lung diseases (CLD) has a heterogeneous presentation and the mechanisms underlying its development and severity are unknown. The study aimed to assess the protein expression profile in plasma in patients with chronic obstructive pulmonary disease (COPD) or fibrosing idiopathic interstitial pneumonia (FIIP), according to the presence and severity of PH, and explore similarities with idiopathic pulmonary arterial hypertension (iPAH).

We analysed 115 patients with CLD: 40 without PH (19 COPD, 21 FIIP), 41 with moderate PH (22 COPD, 19 FIIP) and 34 with severe PH (21 COPD, 13 FIIP); and 38 patients with iPAH. Proteomic abundance data were log2 transformed and differential expression was assessed using unpaired Welch t-statistics. We selected differential expressed proteins when they had p-value <0.05 and presence >80%.

COPD and FIIP patients showed different proteomic profiles. In COPD, inter-alpha-trypsin inhibitor heavy chain-1 (ITIH1), ITIH2, ficolin-3 (FCN3) and vascular cell adhesion molecule-1 (VCAM1) were differentially expressed in PH compared to non-PH, and also in severe PH compared to non-severe PH. These proteins are related to extracellular matrix stabilization and complement pathways. Patients with severe COPD-PH showed 59 proteins differentially expressed, compared to non-severe PH; whereas in FIIP only 11 proteins differed between patients with severe and non-severe PH. Many proteins up-regulated in severe COPD-PH are immunoglobulins, which highlights the importance of the immune system in these conditions.

We conclude that in patients with CLD, the protein expression profile associated with the presence and severity of PH differs between COPD and FIIP. In severe COPD-PH, the expression of proteins related to immunity and extracellular matrix differs from patients with non-severe PH and might serve as a molecular signature of this condition.

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Towards an omic classification of pulmonary hypertension

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Pulmonary arterial hypertension (PAH) is the first of five types of pulmonary hypertension (PH). It is a rare, progressive disease characterized by pulmonary arterial remodeling and increased pulmonary artery pressure, which can lead to right ventricular failure and death if untreated. In 2000, the first gene associated with the development of PAH, *BMPR2*, was discovered, and since then, thanks to advances in next generation sequencing, more than 20 additional genes have been associated.

In this work we have analyzed the genome of patients with different types of PAH, mostly idiopathic pulmonary arterial hypertension (IPAH), with the aim of obtaining a genetic diagnosis through a virtual gene panel and, in negative cases, extending the study to the whole genome in search of new variants involved in the development of the disease. In this case, all patients were negative for the gene panel and the whole genome analysis has allowed us to identify a variant in *VASH1*, which had not previously been associated with the development of PAH. However, it is important to keep in mind that these are only preliminary results.

Consequently, this work supports the importance of the application of whole genome sequencing in the genetic study and diagnosis of rare and severe diseases such as PAH.

Dysfunction of endothelial progenitor cells in pediatric pulmonary hypertension: a characterization study

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In children, pulmonary hypertension (PH) is commonly associated with underlying cardiac or lung disease such as congenital heart disease (CHD) or bronchopulmonary dysplasia (BPD). Endothelial progenitor cells (EPCs) are crucial in vasculogenesis but their role in the progression and reversibility of the disease remains unclear. Therefore, this study aims to characterize the potential dysfunction of EPCs isolated from paediatric patients with or without PH. EPCs were isolated from 11 paediatric patients with different forms of PH and 7 with CHD without PH (non-PH). EPCs were incubated in basal medium or stimulated with serum before assessing proliferation with the MTT assay. IL-6 production was quantified by ELISA. The study is approved by the ethics committees. EPCs from patients with PH (PH-EPCs) did not show a significant increase in proliferative potential compared to non-PH EPCs. However, PH-EPCs exhibited a higher capacity for secreting IL-6 compared to non-PH EPCs. Furthermore, when patients were classified into three groups based on underlying causes of PH, it was found that EPCs from patients with idiopathic or heritable PAH (IPAH) and PAH associated with CHD showed a significant increase in their proliferative potential compared to non-PH-EPCs or EPCs from patients with PH associated with BPD. Additionally, only IPAH-EPCs exhibited a higher capacity for secreting IL-6 compared to non-PH-EPCs or EPCs isolated from patients with other forms of PH. These findings suggest that EPCs from children with PAH may present a hyperproliferative and proinflammatory phenotype but their role may vary based on the underlying causes of PH. This highlights the potential importance of considering the specific aetiologies of PH when studying EPCs and their role in the disease. Further research in this area could provide valuable insights for developing targeted therapeutic approaches for different forms of PH.

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