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Clinical report

Mutational screening in genes related with porto-pulmonary hypertension: An analysis of 6 cases☆

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ABSTRACT

Introduction: Portopulmonary hypertension (PPH) is a rare disease with a low incidence and without a clearly-identified genetic component. The aim of this work was to check genes and genetic modifiers related to pulmonary arterial hypertension in patients with PPH in order to clarify the molecular basis of the pathology.
Patients: We selected a total of 6 patients with PPH and amplified the exonic regions and intronic flanking regions of the relevant genes and regions of interest of the genetic modifiers.
Results: Six patients diagnosed with PPH were analyzed and compared to 55 healthy individuals. Potentially-pathogenic mutations were identified in the analyzed genes of 5 patients. None of these mutations, which are highly conserved throughout evolution, were detected in the control patients nor different databases analyzed (1000 Genomes, ExAC and DECIPHER). After analyzing for genetic modifiers, we found different variations that could favor the onset of the disease.
Conclusions: The genetic analysis carried out in this small cohort of patients with PPH revealed a large number of mutations, with the *ENG* gene showing the greatest mutational frequency.

Cribado mutacional en genes relacionados con la hipertensión portopulmonar: análisis de 6 casos

Palabras clave:
Hipertensión arterial pulmonar
Hipertensión portopulmonar
Mutaciones
Estudio genético
Modificadores genéticos

RESUMEN

Introducción: La hipertensión portopulmonar (HPP) es una enfermedad rara de baja incidencia y sin una alteración genética claramente identificada. El principal objetivo de este estudio fue analizar los genes y modificadores genéticos relacionados con la hipertensión arterial pulmonar en pacientes con HPP.
Pacientes: Se seleccionaron 6 pacientes diagnosticados de HPP y se amplificaron las regiones exónicas y sus límites intrónicos de los genes y la región de interés en los modificadores genéticos.
Resultados: Se analizaron 6 pacientes diagnosticados de HPP y se compararon con 55 individuos sanos. Se identificaron mutaciones potencialmente patogénicas en 5 pacientes en alguno de los genes analizados. Ninguna de estas mutaciones, que se encuentran altamente conservadas a lo largo de la evolución, fue detectada en los controles analizados ni en las diferentes bases de datos consultadas (1000 Genomas, ExAC y DECIPHER). Tras el análisis de los modificadores genéticos encontramos diferentes variaciones que podrían favorecer el desarrollo de la enfermedad.
Conclusiones: El análisis genético en esta pequeña serie de pacientes con HPP ha mostrado un elevado número de mutaciones, siendo el gen *ENG* el que muestra una mayor frecuencia mutacional.

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Introduction

Portopulmonary hypertension (PPH, ORPHA 275813) is considered a clinical entity in itself and is defined as pulmonary arterial hypertension (PAH; OMIM #178600; ORPHA 422) in the presence of portal hypertension from any cause, with or without liver cirrhosis. It is a rare type of PAH, representing just over 5% of PAH,[1] according to the REVEAL registry. For its diagnosis, there must be evidence of portal hypertension and hemodynamic values compatible with PAH. The exact incidence and prevalence are not known. Percentages between 6% and 8% have been observed in studies of patients referred for liver transplant evaluation.[2] The values obtained in the Spanish registry (REHAP) show a percentage close to 6% for PPH on total PAHs, similar to other registries.[3] There are some factors that seem to be associated with the presence of PPH, such as being female or an autoimmune etiology of liver disease, while it is less frequent in cases of hepatitis C virus.[4]

PPH pathogenesis is not fully clarified and histological lesions in pulmonary arteries are indistinguishable from those observed in idiopathic PAH.[2,5] It is possible that stress on the vascular wall caused by increased pulmonary blood flow associated with portal hypertension, in the presence of a favorable genetic load, could cause an alteration in the balance between the various vasoactive substances of the endothelium, promoting the development of the lesions previously described.[6]

Data on the genes involved in this disease are very limited.[6] It has been speculated that the gene encoding the bone morphogenetic protein receptor type II *(BMPR2)* and the gene of kinase-like activin receptor type II *(ALK1/ACVRL1) could be involved*, both related to the pathogenesis of PAH. The *BMPR2* gene is altered in about 80% of patients with heritable PAH and in up to 40% of patients with idiopathic PAH.[7] However, cases of PAH associated with PPH with mutations in these genes have not yet been reported.

The primary objective of this study was the analysis of key genes and different genetic modifiers associated with PAH in patients with PPH, in order to delve into the molecular basis of this disease.

Patient presentation

6 patients were included in this study, and the diagnosis was made based on medical record, imaging tests, liver biopsy and hemodynamic studies. A right heart catheterization was performed in all cases. PAH was considered if there was a mean pulmonary arterial pressure ≥ 25 mmHg and an arterial wedge pressure ≤ 15 mmHg without specific treatment, following the protocol in accordance with the recommendations of the *European Respiratory Society/European Society of Cardiology*. Patients were stable at the time of catheterization. As in previously published studies, samples from 55 healthy individuals who had no known relatives affected by PAH were used as controls.[7]

All patients and controls signed an informed consent, in accordance with the ethical principles for medical research involving human subjects set forth in the Declaration of Helsinki and endorsed by the World Medical Association. The local ethics committee (Autonomic Research Ethics Committee of Galicia) gave its approval for the study.

Of the 6 patients, the etiology of liver disease was autoimmune in one case and alcohol-related in the other 5. At the time of diagnosis 2 patients were in functional class (FC) II, 3 patients in FC III and one in class IV (Fig. 1). The clinical and hemodynamic characteristics of patients are shown in Table 1.

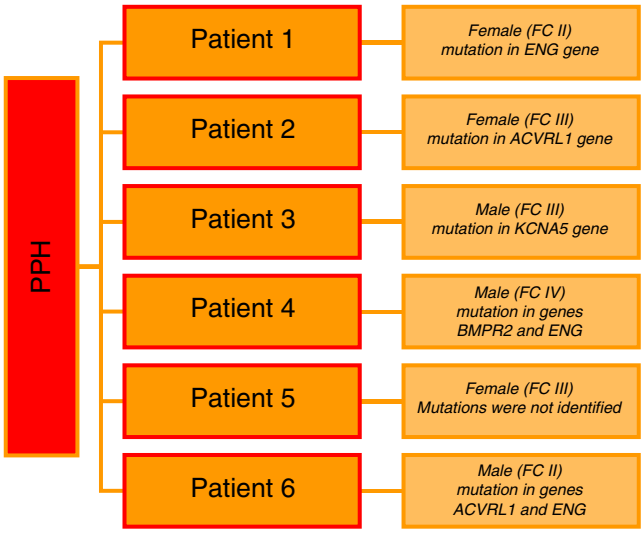

Fig. 1. Characteristics of patients with portopulmonary hypertension included in this study. FC: functional class; PPH: portopulmonary hypertension.

Results

Mutational analysis

After a thorough *in silico* analysis, potentially pathogenic mutations located in the intronic region, or synonymous mutations that can affect mRNA processing, or missense mutations, or frameshift mutations were identified. Through genetic characterization of genes *BMPR2*, *ACVRL1*, endoglin (*ENG*) and K^+ voltage-gated channels, member 5 (*KCNA5*), some potentially pathogenic mutation was identified in 5 of 6patients analyzed (Table 2). None of the mutations classified as pathogenic were detected in the 110-chromosome control panel. These were searched in different databases (1000 Genomes, ExAC and DECIPHER). However, only the mutation c.1633G > A (p.G545S) of the gene *ENG* has been previously described in the ExAC database, being classified as pathogenic. The recommendations of the American College of Medical Genetics and Genomics were followed to classify these variants as pathogenic.[8]

Also, numerous changes classified as polymorphisms have been identified in these patients, and, in one patient, a variation c.572G > A (p.G191N) in the gene *ENG* was identified, and although the *in silico* analysis classifies it as pathogenic, other studies classify it as polymorphism. For this reason, until no other functional studies are conducted, it should be classified as a variant of uncertain significance.

All missense mutations identified in this study are conserved throughout evolution. The exact point of the wild sequence where the study mutation was found was compared to 10 different species.

Analysis of genetic modifiers

The c.1-1853_1897del44 gene polymorphism in the serotonin transporter *(SLC6A4/SERT1)* is present in 4 patients analyzed. For the endothelin-1 *(EDN1)* gene, c.5665G > T polymorphism only appears in 2 patients analyzed.

The 3 polymorphisms studied, associated with the transient receptor potential channel C, isoform 6 *(TRPC6)* gene, are present in some patients. The variation c.1-361A > T appears in 4 patients, and variations c.1-254C > G and c.1-218C > T are present in 3 patients, one of each. The c.1166A > C polymorphism of angiotensin receptor type I*(AGTR1)* gene was identified in 3 patients.

Finally, in 5 of the cases analyzed, the CCTTT repeat polymorphism of inducible nitric oxide synthase *(NOS2)* gene has at least