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Clinical report

Pulmonary arterial hypertension associated to systemic erythematosus: Molecular characterization of 3 cases☆

Guillermo Pousada[a,b,c], Mauro Lago-Docampo[a], Adolfo Baloira[d], Diana Valverde[a,b,*]

[a] Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo, Spain
[b] Instituto de Investigación Biomédica de Ourense-Pontevedra-Vigo (IIS Galicia Sur), Pontevedra, Spain
[c] Centro de Investigaciones Biomédicas (CINBIO) (Centro Singular de Investigación de Galicia), Universidad de Vigo, Pontevedra, Spain
[d] Servicio de Neumología, Complexo Hospitalario Universitario de Pontevedra, Pontevedra, Spain

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ABSTRACT

Background and objective: Pulmonary arterial hypertension associated with systemic lupus erythematosus (PAH-SLE) is a rare disease with a low incidence rate. In this study, PAH related genes and genetic modifiers were characterised molecularly in patients with PAH-SLE.
Patients and methods: Three patients diagnosed with PAH-SLE and 100 control individuals were analysed after signing an informed consent.
Results: Two out of the three analysed patients with PAH-SLE were carriers of pathogenic mutations in the genes *BMPR2* and *ENG*. After an *in silico* analysis, pathogenic mutations were searched for in control individuals and different databases, with negative results, and they were thus functionally analysed. The third patients only showed polymorphisms in the genes *BMPR2*, *ACVRL1* and *ENG*. Several genetic variants and genetic modifiers were identified in the three analysed patients. These modifiers, along with the pathogenic mutations, could lead to a more severe clinical course in patients with PAH.
Conclusions: We present, for the first time, patients with PAH-SLE carrying pathogenic mutations in the main genes related to PAH and alterations in the genetic modifiers.

Hipertensión arterial pulmonar asociada a lupus eritematoso sistémico: caracterización molecular de 3 casos

Palabras clave:
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Mutaciones
Modificadores genéticos
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RESUMEN

Introducción y objetivos: La hipertensión arterial pulmonar asociada a lupus eritematoso sistémico (HAP-LES) es una rara enfermedad de baja incidencia. En este estudio se caracterizaron molecularmente los genes y modificadores genéticos relacionados con la HAP en pacientes con HAP-LES.
Pacientes y métodos: Se analizaron 3 pacientes diagnosticados de HAP-LES y 100 individuos control, previa firma del consentimiento informado.
Resultados: Dos de las 3 pacientes con HAP-LES analizadas resultaron ser portadoras de mutaciones patogénicas en los genes *BMPR2* y *ENG*. Tras el análisis *in silico*, las mutaciones patogénicas se buscaron en individuos control y en diferentes bases de datos, siendo este resultado negativo, por lo que fueron analizadas funcionalmente. La tercera paciente tan solo presentó polimorfismos en los genes *BMPR2*, *ACVRL1* y *ENG*. Se identificaron diversas variaciones en los modificadores genéticos en las 3 pacientes analizadas. La presencia de estos modificadores, junto con las mutaciones patogénicas, podrían dar lugar a un fenotipo más severo en los pacientes con HAP.
Conclusiones: Presentamos, por primera vez, pacientes con HAP-LES portadores de mutaciones patogénicas en los principales genes relacionados con la HAP y con alteraciones en los modificadores genéticos.

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* Corresponding author.
E-mail address: dianaval@uvigo.es (D. Valverde).

Introduction

Pulmonary arterial hypertension (PAH, OMIM # 178600, ORPHA 422) is a rare disease that is inherited in an autosomal dominant manner with incomplete penetrance.[1] It is characterised by the remodelling of the precapillary pulmonary arteries, resulting in a thickening of the vascular walls and increased resistance to blood flow that ends up causing right heart failure and premature death.[1] PAH has an annual incidence of between 1.2 and 3.2 cases per million and a prevalence of between 4.6 and 16 cases per million, being between 2 and 4 times more prevalent in women.[2] The average age at diagnosis is around 45 years, although the symptoms can occur at any age.[2,3]

PAH is classified as idiopathic PAH, familial PAH or associated with other diseases or exposure to drugs or toxins.[1–3] Within PAH associated with other diseases or exposure to drugs or toxins, PAHs associated with connective tissue diseases stand out, with scleroderma and systemic lupus erythematosus (SLE) being the most common.[1–3] It is estimated that PAH associated with SLE (PAH-SLE) affects between 0.5% and 14% of patients diagnosed with SLE.[4,5] Patients with PAH-SLE are young women in 90% of cases, with a mean age at diagnosis of 33 years. The mean time from the diagnosis of SLE to the development of PAH was 4.9 years according to the French registry, with a 5-year survival rate of 83.9%.[4–6]

The genetic mechanisms of PAH have not yet been completely unravelled. Mutations in bone morphogenetic protein receptor type II (*BMPR2*) have been related to more than 70% of familial PAH cases and up to 40% of idiopathic PAH cases. Other genes such as activin receptor-*like*-kinase 1 (*ALK1*/*ACVRL1*) and endoglin (*ENG*) have also been linked to the development of PAH.[7] However, to date, mutations in these genes have been reported in just a few studies in patients with systemic scleroderma.[8] On the contrary, other genes have been related to systemic scleroderma.[9,10]

To date, no mutations have been described in the main genes involved in PAH in patients with PAH-SLE. For this reason, this study analyses the main genes and genetic modifiers related to PAH in 3 patients diagnosed with PAH-SLE.

Patient description

The study included three patients, all women, with PAH-SLE, after signing the informed consent, in accordance with the ethical principles for medical research involving human subjects established in the Declaration of Helsinki and promoted by the World Medical Association. Likewise, the local Ethics Committee (Clinical Research Ethics Committee of Galicia) gave its approval for the study.

The diagnosis of PAH was made by means of a right cardiac catheterization. The diagnostic criteria were a mean pulmonary arterial pressure $\geq$25 mmHg with a pulmonary wedge pressure $\leq$15 mmHg without treatment, according to the protocol established by the *European Respiratory Society* and the *European Society of Cardiology*. Two of the patients were in functional class II and one patient in FC III. An angio-computed tomography was performed in all cases to rule out thromboembolism. For the treatment of PAH, bosentan was used in two cases and ambrisentan in one case. All three patients had positive anticardiolipin antibodies. Raynaud's phenomenon was present in 2 of them. One of the patients was under treatment with mycophenolate and cyclophosphamide and the other two with cyclophosphamide, prednisone and hydroxychloroquine. After 3 years of follow-up, none had died. The clinical and haemodynamic characteristics are shown in Table 1.

In order to evaluate the changes identified in the genes and genetic modifiers related to PAH analysed in this study, samples from 100 healthy general population individuals were used as controls, without known relatives affected by PAH, already used in other studies by our research group.[7]

Results

Some of the main genes related to PAH, such as *BMPR2* (bone morphogenetic protein receptor type II), *ACVRL1* (activin receptor-*like* kinase 1), *ENG* (endoglin) and *KCNA5* (voltage-gated K^+ channels, member 5), were analysed in the 3 patients included in this study. After their identification, an exhaustive *in silico*

Table 1
Clinical and haemodynamic characteristics of patients with PAH associated with lupus included in this analysis.

Clinical and haemodynamic characteristics	Patients with PAH associated with lupus		
	Patient 1	Patient 2	Patient 3
Sex	Female	Female	Female
Functional class	II	III	II
Age at diagnosis (years)	29	38	50
mPAP (mmHg)	45	29	26
SPAP (mmHg)	70	50	45
PVR (mmHg l^{-1} m^{-1})	8	7.5	3.52
CI (lm^{-1} m^{-2})	2.3	2.6	2.8
TM6M (m)	441	450	450
Treatment for PAH	Bosentan	Bosentan	Ambrisentan
Raynaud's syndrome	Yes	Yes	No
Anti-Ro/SS-A antibodies	+	+	+
Anti-RNP antibodies	+	+	–
Anti-CL antibodies	+	+	+
Treatment for SLE	Mycophenolate and cyclophosphamide	Cyclophosphamide, prednisone and hydroxychloroquine	Cyclophosphamide, prednisone and hydroxychloroquine
Death (3 years)	No	No	No

Anti-CL: anticardiolipin antibodies; Anti-RNP: anti-ribonucleoprotein antibodies; Anti-Ro/SS-A: anti-DNA antibodies; CI: cardiac index; mPAP: mean pulmonary arterial pressure; SPAP: systolic pulmonary arterial pressure; PVR: pulmonary vascular resistance; TM6M: 6-minute walk test.