Original Article

Polymorphisms in the Serotonin Transporter Protein (SERT) Gene in Patients With Pulmonary Arterial Hypertension

Adolfo Baloira, a,∗ Marta Núñez, a José Cifrian, b Carlos Vilariño, c María Ojeda, d Diana Valverde d

a Servicio de Neumología, Complexo Hospitalario de Pontevedra, Pontevedra, Spain
b Servicio de Neumología, Hospital Universitario Valdecilla, Santander, Spain
c Servicio de Neumología, Hospital Xeral de Vigo, Vigo, Pontevedra, Spain
d Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo, Pontevedra, Spain

A R T I C L E   I N F O

Article history:
Received 4 July 2011
Accepted 19 October 2011
Available online 28 January 2012

Keywords:
Serotonin
Pulmonary arterial hypertension
Serotonin transporter protein (SERT)
Polymorphisms

A B S T R A C T

Serotonin is a potent vasoconstrictor and pulmonary vascular growth factor whose concentration is increased in patients with pulmonary arterial hypertension (PAH). Its functions are mediated in part by the serotonin transporter protein (SERT) whose gene can have two allelic forms, both long (L) and short (S). The first was associated with greater function.

Objectives: To determine whether the prevalence of the L allelic form of SERT is higher in patients with PAH than in the general population. To observe whether there are any clinical differences in patients with PAH based on the SERT allele.

Methods: We included patients diagnosed with PAH with catheterization based on the established criteria. Peripheral blood samples were taken and the DNA was extracted from the peripheral leukocytes. We amplified the promoter region of SERT by polymerase chain reaction and separated the products by electrophoresis. The patient samples were compared with samples from 50 healthy controls and among the most common types of PAH (idiopathic, thromboembolic and associated with connective tissue disorders). Several clinical variables were assessed according to the SERT gene alleles.

Results: The study included 50 patients, and adequate samples were obtained in 49 patients (30 women). Mean age at diagnosis was 56 ± 16 years. No differences were seen in the distribution of alleles between patients and controls ( P = .54). There were no differences among the three most common types of PAH ( P = .3). The most frequent allelic form was LS (54% patients, 56% controls). There were no differences in either age of diagnosis or response to treatment according to the SERT alleles. There was a trend toward higher pulmonary pressure levels in the LL forms (49 ± 5 mmHg vs 42 ± 9 mmHg, P = .07).

Conclusions: The distribution of SERT gene alleles does not appear to be different in patients with PAH than in the normal population. Different types of PAH have a similar distribution of alleles. The LL forms do not appear to confer either clinical differences or differences in response to treatment.

© 2011 SEPAR. Published by Elsevier España, S.L. All rights reserved.

Polimorfismos en el gen de la proteína transportadora de serotonina (SERT) en pacientes con hipertensión arterial pulmonar

R E S U M E N

La serotonina es un potente vasoconstrictor y factor de proliferación vascular pulmonar cuya concentración se incrementa en pacientes con hipertensión arterial pulmonar (HAP). Sus funciones están mediadas en parte por la proteína transportadora de serotonina (SERT), cuyo gen puede presentar dos formas alélicas, una larga (L) y otra corta (S). La primera se ha asociado a mayor función.

Objetivos: Conocer si la prevalencia de la forma alélica L del gen de SERT es mayor en pacientes con HAP que en población general. Ver si existe alguna diferencia clínica en los pacientes con HAP en función del alelo SERT.

Métodos: Se incluyeron pacientes diagnosticados de HAP con cateterismo en base a los criterios establecidos. Se extrañó una muestra de sangre periférica y posteriormente se extrajo DNA de los leucocitos periféricos.
Se amplificó la región promotora de SERT mediante reacción en cadena de polimerasa y se separaron los productos mediante electroforesis. Se compararon las muestras de los pacientes con 50 controles sanos y entre los tipos más frecuentes de HAP (idiopática, tromboembólica y asociada a connotipatías). Se valoraron diversas variables clínicas en función de los diversos alelos del gen SERT.

**Resultados:** Se incluyó a 50 pacientes, y se obtuvo muestra adecuada en 49 (30 mujeres). La edad media en el momento del diagnóstico fue 56 ± 16 años. No se observaron diferencias en la distribución de alelos entre pacientes y controles (p = 0,54). Tampoco existieron diferencias entre los tres tipos más frecuentes de HAP (p = 0,3). La forma más frecuente fue LS (54% pacientes, 56% controles). Tanto la edad de diagnóstico como la respuesta al tratamiento no fueron diferentes en función de los alelos SERT. Hubo una tendencia a presentar mayores valores de la presión pulmonar media en las formas LL (49 ± 5 vs. 42 ± 9 mmHg, p = 0,07).

**Conclusions:** La distribución de los alelos del gen SERT no parece ser diferente en los pacientes con HAP de cómo se presenta en la población normal. Diversos tipos de HAP tienen una distribución de alelos similar. Las formas LL no parecen conferir diferencias clínicas ni de respuesta al tratamiento.

© 2011 SEPAR. Publicado por Elsevier España, S.L. Todos los derechos reservados.
Table 1
Types of Pulmonary Arterial Hypertension (PAH).

<table>
<thead>
<tr>
<th>Type of PAH</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic</td>
<td>14</td>
</tr>
<tr>
<td>Thromboembolic</td>
<td>14</td>
</tr>
<tr>
<td>Connective tissue pathologies</td>
<td>13</td>
</tr>
<tr>
<td>Congenital heart disease</td>
<td>4</td>
</tr>
<tr>
<td>HIV</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
</tr>
</tbody>
</table>

HIV: human immunodeficiency virus.
The three most frequent types of PAH in our patients were idiopathic, thromboembolic and associated with connective tissue disorders. "Other" includes one portopulmonary hypertension and one case associated with chronic obstructive pulmonary disease.

Table 2
Distribution of the SERT Alleles in Patients and Healthy Controls.

<table>
<thead>
<tr>
<th></th>
<th>LL</th>
<th>LS</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>10</td>
<td>26</td>
<td>13</td>
</tr>
<tr>
<td>Controls</td>
<td>15</td>
<td>23</td>
<td>12</td>
</tr>
</tbody>
</table>

The distribution of the 3 alleles did not show differences between patients and controls (P=.54). When we compared the LL vs non-LL alleles, there were also no differences (P=.27). In both groups, the LS type was the most frequent.

categorical variables were analyzed using the Chi-squared test, and a value equal to or less than 0.05 was considered significant. All the analyses were done with the SPSS statistical program, version 15.0 (SPSS, Inc., Chicago, IL, USA).

Results

Out of the 50 patients included in the study, in 49 patients, it was possible to correctly analyze polymorphism. Of these, 14 corresponded with idiopathic PAH, 13 PAH associated with connective tissue pathologies, 14 chronic thromboembolic disease, 4 cases were related with congenital cardiopathy, 2 were associated with human immunodeficiency virus, one case PAH with portal hypertension and another with chronic obstructive pulmonary disease (Table 1). Mean age at the time of diagnosis was 56±16 (range, 25–78 years); there were 30 women and 19 men.

The distribution of SERT genotypes did not vary between the control group and patients (LL: 20% vs 30%, P=.54) (Table 2). We also did not observe any differences between the three predominate types of PAH (P=.3) (Table 3). The most frequent genotype was LS (54% of patients, 56% of controls), and although within the group of patients a greater frequency of LL genotype was observed in men than in women (25% vs 14.8%), statistical significance was not reached (P=.33).

There were no differences in the age at diagnosis (LL: 57±13 vs 57±12, P=.56). A tendency was observed toward presenting higher PAPlv levels in patients with the LL genotype, although without reaching statistical significance (49±5 mmHg vs 42±9 mmHg, P=.07). In 34 patients, the follow-up period lasted at least one year. In 18 cases, there was a good response to treatment. The distribution of LL or non-LL types did not vary according to whether they were responders or not (P=.8). Only 3 deaths occurred during the follow-up period, all of them patients with non-LL genotypes.

Discussion

In our series of patients, the distribution of SERT gene alleles was similar to that found in healthy controls. There were no observed differences between the three most frequent types of PAH or between sexes. Moreover, the presence of LL was not associated with a different behavior of the disease in general, despite a certain tendency toward having a higher level of PAPlv, a parameter which in the majority of studies does not correlate with survival.10,11 The presence of one type or another of SERT genotypes also did not influence the mid-term therapeutic response.

There are several studies that seem to demonstrate a very important role of serotonin in the pathway of PAH.12 Some years ago, the “serotonin hypothesis of PAH” was described because the mechanism of action of some appetite suppressant drugs (such as anorex and dexfenfluramine) linked to the appearance of this disease inhibited the neuronal recapitulation of serotonin and, therefore, greater secretion and stimulation of its receptors.13 Serotonin stimulates the proliferation of smooth muscle cells and fibroblasts of the pulmonary vessels and, when cultured with it, these cells extracted from patients with PAH proliferate with much greater intensity than those of healthy controls.14 The serotonin pathway is complex and is influenced by its synthesis, receptors and the SERT protein.15 Some years ago, a discovery was made of the existence of a variant in the promoter region of the SERT gene, with a long allele that increased the transcription compared with the other shorter one. An initial study showed a greater prevalence of the LL genotype in patients with PAH.7 Nevertheless, other studies with larger patient numbers did not find differences with the general population. A French-American study including 166 patients with hereditary PAH and 83 with idiopathic PAH did not observe differences in the distribution of the three SERT genotypes compared with 125 healthy controls. The LS type, as in our patients, was the most frequent. The only relevant finding was the appearance of the disease at a younger age in the cases of hereditary PAH if they were carriers of the LL genotype.16 In those who presented idiopathic PAH, the age at onset was similar, something which also happened in our series. The results did not vary when we analyzed the presence of mutations in the type 2 receptor of the bone morphogenetic proteins (BMPR2). In our study, only 3 patients of the 9 in whom we studied this gene were carriers of a mutation; therefore, we could not make comparisons. There were no survival differences depending on the SERT genotype. In our series, we were only able to analyze the response to treatment in 34 patients (70%) because in the remainder the follow-up was less than one year, with no differences found between genotypes. As only three deaths occurred, it was not possible to make a survival analysis depending on the SERT genotype. Another study by Machado et al.17 on a wide patient base that included familiar, idiopathic and associated types also found no differences in the distribution of the SERT genotypes. In this case, the age at onset of the disease in the familiar forms did not show differences with the non-familiar ones, which contrasts with the former study. As in our case, a comparison by sexes also did not present different distribution of the SERT genotypes. It is possible that in some specific types of PAH the SERT protein may have a more relevant role. One previously commented article done in France and the United Kingdom, which included 103 patients diagnosed with severe chronic obstructive pulmonary disease (mean FEV1 37% predicted), found no differences with the controls in the distribution of the SERT genotypes. However, the most important finding was that the disease was more severe in those who carried the LL type than the LS or SS types (PAPlv 34±3, 23±1 and 22±1 mmHg, respectively, P<.01).5 In part, this corresponds with our results.
although in this case without reaching statistical significance. In this study, the expression of messenger RNA for serotonin in the muscle cells of the pulmonary arteries was more than double in those patients with LL types than in those who had any other type and, at the same time, if subjected to hypoxia, a powerful stimulant of serotonin, the LL types had 5 times more expression than the normoxic SS types. All these data support the important role that serotonin and its action pathways may have in the genesis of the underlying lesions in PAH.

The contribution of the LL genotype of SERT to favoring/aggravating PAH is difficult to quantify. It is very likely that several genetic factors are involved in the development of the disease, and quite probably linked with environmental factors. It is not easy to design studies about possible candidate genes that take into account the majority of the exogenic variables that could influence the appearance and the evolution of PAH. This, together with the low prevalence of this disease, which makes it difficult to include a high number of patients, can lead to significant biases with results that are difficult to interpret. For these reasons, we believe it is very important to continue to carry out genetic studies in order to establish risk profiles and open new therapeutic pathways. With the data available, it does not seem that this polymorphism in the SERT gene plays a relevant role in the appearance of PAH. Perhaps the LL types could be an aggravating factor of the disease in some types of patients, especially if associated with hypoxia, which should be explored.

One of the most important limitations of our study is the small number of patients. As we have already commented, PAH is a disease with a low prevalence (estimated at about 15 cases per million inhabitants\(^)\), which makes it difficult to bring together an extensive sample. It is possible that the results would be different if the number of patients were greater, but the coincidence of our findings with those of the most recent publications confer them greater credibility. Compared with the other larger series analyzing the samples of different countries with difficulty for obtaining data in an important number of cases, in our study we had all the clinical information made available to us.

In conclusion, despite the evidence that confer the SERT gene (especially its LL type) an important role in the action of serotonin as a possible candidate for increasing the susceptibility for developing PAH, we have not been able to demonstrate a greater number of patients who are carriers for this allele compared with the general population, nor was there a clear association with disease severity.

### Funding

This study has been completed thanks to a research grant from Actelion.

### Conflict of Interests

The authors declare having no conflict of interests.

### References