



Original Article

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

Adolfo Baloira,^{a,*} Marta Núñez,^a José Cifrián,^b Carlos Vilariño,^c María Ojeda,^d Diana Valverde^d

^a Servicio de Neumología, Complejo 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

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ABSTRACT

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 mean 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.

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

RESUMEN

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 extrajo una muestra de sangre periférica y posteriormente el ADN de los leucocitos periféricos.

Palabras clave:

Serotonina

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* Corresponding author.

E-mail address: adolfo.baloira.villar@sergas.es (A. Baloira).

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 conectivopatías). 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, $p = 0,07$).

Conclusiones: 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.

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Introduction

Pulmonary arterial hypertension (PAH) is a severe disease in which there is a progressive increase in pulmonary vascular resistance while the left heart pressure remains normal. Although the pathogenesis of the disease is not understood, the research that has taken place over recent years has revealed some of the cellular pathways involved in the development of characteristic PAH lesions. In 2000, one of the most important findings came about when it was revealed that the genetic alteration responsible for the hereditary form was situated in the gene that codifies BMPR2, one of the bone morphogenetic protein receptors.^{1,2} Since then, there has been an intense search for possible genes related with vascular regulation. Serotonin plays a crucial role, and it also has an important stimulating effect on the proliferation of smooth muscle cells of the pulmonary vasculature.³ Some years ago, an increase was reported in the plasma concentration of serotonin in 16 patients with idiopathic PAH.⁴ This may occur in two manners: uniting with specific receptors of the cell membrane or entering into the cytoplasm thanks to the action of the serotonin transporter protein (SERT). This latter mechanism of action is responsible for the proliferative effects.⁵

The SERT gene is located in chromosome 17 and has 14 exons totaling 31 kilobases. The promoter region of the gene may have two variants, one long (L) and another short (S), situated at a distance of one kilobase from the transcription initiation sites, which consist of the insertion (L forms) or the deletion (S forms) of 44 pairs of bases. The presence of the L allele is associated with a transcriptional activity between 2 and 3 times higher than that of the S allele. In patients with chronic obstructive pulmonary disease, the LL allele correlated with higher levels of pulmonary arterial pressure.⁶ There is a certain disparity of the results in the small number of studies performed on the distribution of the SERT genotypes in patients with PAH compared with the general population, as well as its impact in the manner of presentation and evolution of the disease. One study seems to show an increase in the LL types.⁷ The objective of our study was to know whether the patients with PAH could have a higher prevalence of this LL genotype and if it is related with the severity of the disease.

Material and Methods

The study population consisted of 50 consecutive patients diagnosed with PAH, both idiopathic as well as secondary, and 50 healthy controls (25 women and 25 men) randomly selected from the Blood Transfusion Center of Galicia. The diagnosis of PAH was based on right catheterization with a mean pulmonary arterial pressure (PAPm) ≥ 25 mmHg and a wedge pressure less than 15 mmHg without specific treatment. In all cases, the protocol for

standard management was followed in accordance with the recommendations of the ERS/ESC.⁸ The patients were stable at the time of the catheterization. The patients as well as the controls were informed in detail about the study, and they gave their written consent to participate. The local ethics committee approved the study.

Laboratory Studies

Blood was extracted and the DNA of the peripheral leukocytes was obtained using the FlexiGene DNA kit by Qiagen (Germany). For the amplification of 50ng of DNA by means of polymerase chain reaction (PCR) of the promoter region, the following primers were used:

5'GGCGTTGCCGCTCTGAATGC3'
5'GAGGGACTGAGCTGGACAACCAC3'

The amplification was carried out in a medium with 1.5 mM of $MgCl_2$, with 0.2 U of Taq polymerase (Biotaq, Bioline, United Kingdom) and 35 cycles of denaturation at 95 °C for 1 min, annealing at 65 °C for 1 min and extension at 72 °C for 1 min. The products of the amplifications were separated with electrophoresis in a 2% agarose gel stained with ethidium bromide. Of the fragments obtained, the one with 484 base pairs (bp) corresponded with the S allele, while the fragment of 528 bp corresponded with the L allele. Finally, electrophoresis was carried out of the amplified fragments (S and L alleles).

Correlation of Genotype/Symptoms

The primary objective was to know the prevalence of the LL genotype in the patients with PAH compared with healthy controls. Due to previous studies, it seems that only the LL types have a significant increase in the transporting action of SERT⁹; therefore we compared this genotype with the other two (LS and SS), which seem to be similar in this aspect. In order to determine the impact of the presence of this polymorphism in the clinical presentation, we compared age at diagnosis, PAPm levels and therapeutic response according to SERT genotype. Thus, we evaluated the patients who reached at least one year of follow-up from the start of the treatment. A good response was considered as either an increase greater than 50 m in the distance walked in the 6-min walk test or an improvement of at least one functional class 12 months after the start of treatment. Given the small number of deaths, it was not possible to calculate survival curves.

Statistical Analysis

All the values are expressed as mean \pm SD. Given the sample size, the means were compared using a non-parametric test (Wilcoxon). The catheterization was considered the time of diagnosis. The

Table 1
Types of Pulmonary Arterial Hypertension (PAH).

Type of PAH	Cases
Idiopathic	14
Thromboembolic	14
Connective tissue pathologies	13
Congenital heart disease	4
HIV	2
Other	2

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.

	LL	LS	SS
Patients	10	26	13
Controls	15	23	12

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 also were 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 predominant 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 PAPm 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

Table 3
Distribution of the SERT Alleles in the Three Most Frequent Types of Pulmonary Arterial Hypertension (PAH).

	LL	LS	SS
Idiopathic	2	8	4
Thromboembolic	5	5	4
Connective tissue disorders	1	9	3

No differences were observed in the distribution of the SERT alleles among the three predominant types of PAH ($P=.3$).

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 PAPm, 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 pathogeny 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 aminorex and dexfenfluramine) linked to the appearance of this disease inhibited the neuronal recaptation 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 (BMP2). 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 (PAPm 34 ± 3 , 23 ± 1 and 22 ± 1 mmHg, respectively, $P<.01$).⁶ 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 inhabitants¹⁸), 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.

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Conflict of Interests

The authors declare having no conflict of interests.

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