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Plasma Levels of S100A4 in Portopulmonary Hypertension

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Abstract

We previously showed that a single nucleotide polymorphism in S100A4 was associated with developing portopulmonary hypertension (PPHTN) in patients with advanced liver disease. We aimed to determine the association between plasma levels of S100A4 and PPHTN.

We performed a case-control study of patients with advanced liver disease. Cases with PPHTN had mean pulmonary artery pressure > 25 mm Hg, pulmonary vascular resistance > 240 dynes-sec · cm⁻⁵, and pulmonary capillary wedge pressure ≤ 15 mm Hg. Controls with liver disease had right ventricular systolic pressure < 40 mm Hg and normal right atrial and ventricular morphology by echocardiography. Plasma samples were assayed for S100A4.

The study sample included 14 cases with PPHTN and 32 liver disease controls. The mean age for both cases and controls was 52 ± 9 yrs. Eighty percent of cases were female compared to 42% of controls (p = 0.02). There was no difference in S100A4 levels between cases and controls (p = 0.53). Both groups had significantly higher S100A4 levels than healthy volunteers (p < 0.05).

There was no significant difference in plasma levels of S100A4 between PPHTN patients and controls with liver disease, although liver disease itself was associated with increased S100A4 levels.

Keywords

portal hypertension; pulmonary arterial hypertension; genetic susceptibility; S100A4

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Introduction

Experimental models of pulmonary arterial hypertension (PAH) have implicated numerous genes in its pathogenesis. One such gene is S100A4, an intracellular calcium binding protein which causes pulmonary vascular remodeling in transgenic mice over-expressing the gene product (1). S100A4 also plays a role in tissue remodeling in lung and liver fibrogenesis (2, 3) and metastatic tumor invasion (4).

PAH associated with portal hypertension, also known as portopulmonary hypertension (PPHTN), shares many pathological features with idiopathic and familial PAH (5). Approximately 5% of patients with cirrhosis being evaluated for liver transplantation have PPHTN (6–8), which may increase morbidity and mortality and greatly complicate liver transplantation (9,10).

There have been few studies of circulating biomarkers which characterize patients with PPHTN. We have previously shown that genetic variability in a S100A4 single nucleotide polymorphism (SNP) is associated with the risk of PPHTN in patients with liver disease (11). In the current study, we aimed to determine whether plasma levels of S100A4 were higher in patients with PPHTN compared to patients with advanced liver disease without evidence of PPHTN and disease-free controls.

Methods

Study Design and Study Sample

The Pulmonary Vascular Complications of Liver Disease (PVCLD) study enrolled a cohort of 536 patients evaluated for liver transplantation or pulmonary hypertension at seven centers in the United States between 2003 and 2006. The only inclusion criterion was the presence of clinical portal hypertension with or without intrinsic liver disease. We excluded patients with evidence of active infection or recent (< two weeks) gastrointestinal bleeding, or who had undergone liver or lung transplantation. Full details of the cohort are published elsewhere (12).

We performed a case-control study within the PVCLD cohort. The study sample included newly-referred patients who were evaluated with transthoracic echocardiography (routinely performed for pre-transplant evaluation) during the study period. “Prevalent” patients who met the case definition (see below) were also included. We excluded patients with significant obstructive or restrictive lung disease, as previously defined (12). The study sample also excluded patients with human immunodeficiency virus infection (which may independently cause PAH), hepatocellular carcinoma or hepatic mass (which may affect S100A4 levels), or the presence of more than moderate aortic or mitral stenosis or regurgitation or significant left ventricular dysfunction by transthoracic echocardiography (which may result in pulmonary hypertension).

Case and Control Definitions

Cases with PPHTN met the following criteria at entry into the cohort: 1) mean pulmonary artery pressure > 25 mm Hg, pulmonary capillary wedge pressure (or left ventricular end-diastolic pressure) ≤ 15 mm Hg, and pulmonary vascular resistance > 240 dynes·s·cm⁻⁵ measured by right heart catheterization, and 2) no other etiology for pulmonary hypertension. Controls met the following echocardiographic criteria at entry into the cohort: 1) right ventricular (RV) systolic pressure < 40 mm Hg (if estimable) and 2) absence of right atrial or ventricular dilation, hypertrophy, or dysfunction. “Prevalent” cases who had previously undergone evaluation and were subsequently being treated (n = 2) were also included; data

from the initial evaluation (before treatment) were used for these cases. It was recommended that patients with RV systolic pressure > 50 mm Hg with abnormal RV morphology by echocardiography undergo right heart catheterization.

Assays

Plasma was isolated from centrifuged whole blood and stored at -80°C . Plasma was then thawed and S100A4 concentration was assayed by enzyme linked immunosorbent assay (ELISA) (CircuLex, Nagano, Japan). All samples were assayed in duplicate according to manufacturer's instructions (13). Plates were read on a microplate reader and analyzed with SoftMax (Molecular Devices, Toronto, Canada) for quantification. Five samples had repeat analysis for estimation of the intraclass correlation coefficient (ICC) with 95% confidence interval (95% CI). Details of S100A4 genotyping are provided elsewhere (11).

Data Analysis and Sample Size Calculation

We used Student's t-tests, Wilcoxon rank sum tests, chi-square tests, Fisher's exact tests, and Spearman's rho, as appropriate. Fourteen cases with 32 controls provided 80% power to detect a difference in plasma S100A4 levels of 0.9 standard deviations with a logistic non-parametric adjustment ($\alpha = 0.05$) (14).

Results

Fourteen cases with PPHTN and 32 controls with liver disease were assayed for plasma S100A4 level and genotyped. The baseline demographics of the patients are shown in Table 1. The mean $\pm$ SD age for both cases and controls was 52 ± 9 yrs. Eighty percent of cases were female compared to 42% of controls ($p = 0.03$). There was no significant difference in the proportions of non-Hispanic whites between the two groups ($p = 0.16$). One patient with PPHTN was receiving bosentan and one patient was receiving intravenous epoprostenol; all other PPHTN cases were untreated at the time of assessment.

The median plasma S100A4 level was 83 ng/ml (IQR 25-271 ng/ml) for cases compared to 84 ng/ml (IQR 12-156 ng/ml) for controls ($p = 0.58$) (Figure 1A). Both groups had significantly higher S100A4 levels than healthy volunteers without liver disease ($p < 0.05$). These findings did not change significantly after adjustment for gender (Data not shown). Inter-assay reliability for plasma S100A4 was excellent (ICC = 0.99, 95% CI 0.98–1.0).

We then assessed the relationship of plasma S100A4 levels with genotype of the SNP rs743687 within the S100A4 locus. The median plasma S100A4 levels were 544 ng/ml in the one patient homozygous for the minor allele (CC), 147 ng/ml (IQR 19–405 ng/ml) in the eight heterozygous patients (CG), and 74 ng/ml (IQR 18–123 ng/ml) in the 53 patients without the minor allele (GG), consistent with a possible relationship between genotype and plasma S100A4 level ($p = 0.11$). (Figure 1B)

Discussion

This is the first study to examine plasma S100A4 levels in patients with PPHTN and liver disease. While there was no significant difference in S100A4 levels between cases with PPHTN and liver disease controls, both groups had significantly higher levels than healthy controls without liver disease. We examined plasma level according to SNP genotype from S100A4 which suggested that the copy number of the minor allele might correlate with the plasma S100A4 levels.

The mechanism between S100A4 and PAH in animal models and humans is not clear. Merklinger *et al* showed that transgenic mice overexpressing S100A4 displayed increased

thickness of the internal and external lamina of the pulmonary arteries (15). The elevation in RV systolic pressure seen in response to hypoxia failed to reverse with nitric oxide in the transgenic mice. The lungs of these mice showed upregulation of fibulin-5 and elastin expression, which are essential components of the extracellular matrix that contribute to vascular remodeling (16).

SNPs within the S100A4 locus have not been assessed in regards to their effect on the activity or expression of the gene product. We showed that the level of S100A4 might correlate with the SNP genotype associated with PPHTN, although concurrent processes such as hemolysis or inflammatory changes could also raise the plasma S100A4 levels (17,18).

We assessed the value of plasma S100A4 as a biomarker of disease and found no significant differences between liver disease patients with or without PPHTN. However, the presence of liver disease itself likely led to increased plasma S100A4, which has been shown to be a robust marker of hepatic fibrosis (2). Indeed, it is possible that the elevated plasma S100A4 levels which characterize patients with advanced liver disease could serve as the milieu which permits other clinical, environmental, neurohormonal or genetic modifiers to trigger the occurrence of PAH in this setting.

There are limitations to our study. Despite having assembled a multicenter cohort of PPHTN patients, it remains a relatively rare disease leading to a small sample size. However, this study was adequately powered to detect a less than one standard deviation difference between the groups. It is possible that cases may have been misclassified as controls based on echocardiography without confirmatory right heart catheterization. To minimize this possibility, we set very stringent echocardiographic criteria for controls which essentially rule out PPHTN in patients with endstage liver disease.(19). We only examined the correlation of one SNP within the S100A4 locus with plasma levels. It may be important to define haplotypes which could have more substantial associations with the molecular phenotype.

In summary, there were no differences in plasma S100A4 levels between PPHTN patients and controls, which were greater than those of healthy controls. Our study showed that there might be a correlation between genotype variation and plasma S100A4 level. The presence of liver disease may nonspecifically increase plasma S100A4 or the high circulating levels of S100A4 in patients with advanced liver disease could facilitate the development of PPHTN.

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Appendix

The Pulmonary Vascular Complications of Liver Disease Study Group also includes: Columbia University: Jeffrey Okun, Daniel Rabinowitz, Evelyn M. Horn, Lori Rosenthal, Sonja Olsen, Jenna Reinen; Mayo Clinic: Vijay Shah, Russell Wiesner, Linda Stadheim; University of Alabama: J. Stevenson Bynon, Devin Eckhoff, Harpreet Singh, Rajasekhar Tanikella, Keith

Wille, Dorothy Faulk; University of Colorado: Lisa Forman, David Badesch, Ted Perry; The University of North Carolina at Chapel Hill: Roshan Shrestha, Carrie Nielsen, Steven Zacks; University of Pennsylvania: Vivek Ahya, Harold Palevsky, Rajender Reddy, Michael Harhay, Sandra Kaplan; University of Southern California: Neil Kaplowitz, James Knowles.

A.

B. **Figure 1.**

S100A4 level according to disease status (A) and genotype (B). Genotypes include one patient homozygous for the minor allele (CC), patients heterozygous for the minor allele (CG), and patients without the minor allele (GG). Black bar represents median plasma concentration. White box represents interquartile range.

Table 1

Demographics and liver disease variables

Variable	Cases (N=14)	Controls (N=32)	p value
Age, years	52 ± 9	50 ± 10	0.52
Gender, female	11 (79%)	14 (44%)	0.03
Race/Ethnicity			0.16
Non-Hispanic white	13 (93%)	22 (69%)	
Hispanic white	0	6 (19%)	
Other	1 (7%)	4 (13%)	
Etiology of cirrhosis/portal hypertension			
Alcohol	5 (36%)	17 (53%)	0.28
Hepatitis C infection	3 (21%)	17 (53%)	0.046
Autoimmune hepatitis	5 (36%)	2 (6%)	0.02
Non-alcoholic fatty liver disease	0	3 (9%)	0.54
Hepatitis B infection	1 (7%)	1 (3%)	0.52
Primary sclerosing cholangitis	0	2 (6%)	1.0
Primary biliary cirrhosis	2 (14%)	0	0.09
Cryptogenic cirrhosis	1 (7%)	3 (9%)	1.0
MELD score	12 ± 3	13 ± 5 (N=31)	0.65

Data are shown as mean ± standard deviation or n (%). MELD = Model for End-stage Liver Disease