Biomarkers, 2009; 14(3): 156–160

informa
healthcare

RESEARCH ARTICLE

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 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 mmHg, pulmonary vascular resistance >240 dynes s cm^{-5} and pulmonary capillary wedge pressure ≤15 mmHg. Controls with liver disease had right ventricular systolic pressure <40 mmHg 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 controls with liver disease. There was no difference in mean age between cases and controls ($p = 0.52$). Seventy-nine percent of cases were female compared with 44% of controls ($p = 0.03$). There was no difference in S100A4 levels between cases and controls ($p = 0.58$). 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*

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 remodelling in transgenic mice overexpressing the gene product (Greenway et al. 2004). S100A4 also plays a role in tissue remodelling in lung and liver fibrogenesis (Lawson et al. 2005, Rygiel et al. 2008) and metastatic tumour invasion (Garrett et al. 2006).

PAH associated with portal hypertension, also known as portopulmonary hypertension (PPHTN), shares many pathological features with idiopathic and familial PAH (Simonneau et al. 2004). Approximately 5% of patients with cirrhosis being evaluated for liver transplantation have PPHTN (Colle et al. 2003, Kawut et al. 2005, Krowka et al. 2006), which may increase morbidity and mortality

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(Received 29 September 2008; revised 19 January 2009; accepted 22 January 2009)

ISSN 1354-750X print/ISSN 1366-5804 online © 2009 Informa UK Ltd
DOI: 10.1080/13547500902773896

and greatly complicate liver transplantation (Krowka et al. 2004, Sussman et al. 2006).

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 (Roberts et al. 2009). In the current study, we aimed to determine whether or not plasma levels of S100A4 were higher in patients with PPHTN compared with 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 centres 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 (<2 weeks) gastrointestinal bleeding, or who had undergone liver or lung transplantation. Full details of the cohort are published elsewhere (Kawut et al. 2008).

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 pretransplant 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 (Kawut et al. 2008). 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 mmHg, pulmonary capillary wedge pressure (or left ventricular end-diastolic pressure) ≤15 mmHg and pulmonary vascular resistance >240 dynes s cm^{-5} measured by right heart catheterization, and (2) no other aetiology for pulmonary hypertension. Controls met the following echocardiographic criteria at entry into the cohort: (1) right ventricular (RV) systolic pressure <40 mmHg (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 mmHg 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 the manufacturer's instructions (http://www.mblintl.com/mbli/catalog/pdf/CY-8059.pdf). 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 (CI). Details of *S100A4* genotyping are provided elsewhere (Roberts et al. 2009).

Data analysis and sample size calculation

We used Student's *t*-tests, Wilcoxon rank sum tests, χ^2 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 ($\alpha=0.05$) (Hintz 2005).

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. There was no difference in mean age between cases and controls ($p=0.52$). Seventy-nine percent of cases were female compared with 44% of controls ($p=0.03$). There was no significant difference in the proportions of non-Hispanic white subjects 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^{-1} (interquartile range (IQR) 25–271 ng ml^{-1}) for cases compared with 84 ng ml^{-1} (IQR 12–156 ng ml^{-1}) for controls ($p=0.58$)

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%)	
Aetiology 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.

(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). Interassay 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 the genotype of the SNP rs743687 within the S100A4 locus. The median plasma S100A4 levels were 544 ng ml^{-1} in the one patient homozygous for the minor allele (CC), 147 ng ml^{-1} (IQR 19–405 ng ml^{-1}) in the eight heterozygous patients (CG) and 74 ng ml^{-1} (IQR 18–123 ng ml^{-1}) in the 37 patients without the minor allele (GG), consistent with a possible relationship between the genotype and the plasma S100A4 level (p = 0.11) (Figure 1B).

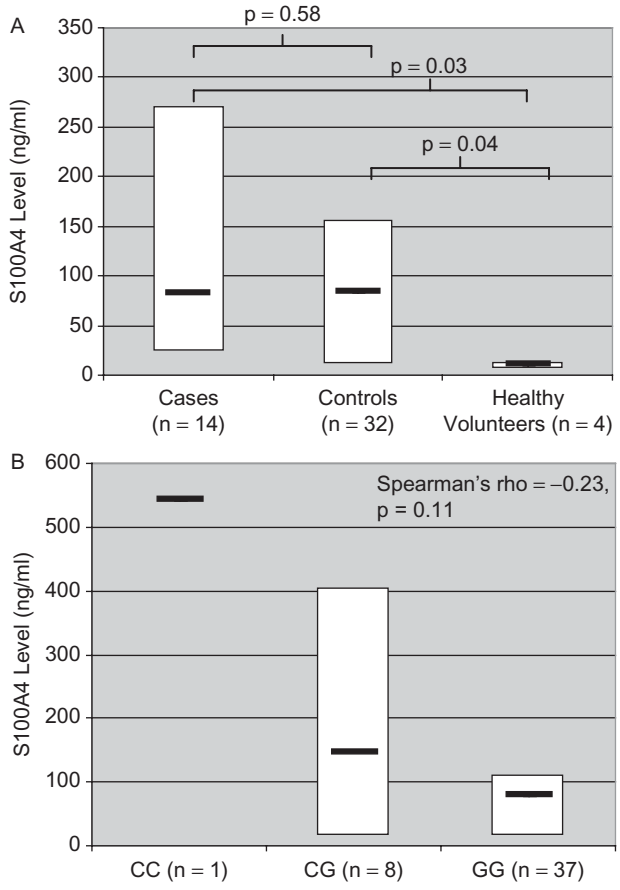

Figure 1. S100A4 level according to disease status (A) and genotype (B). Genotypes include one patient homozygous for the minor allele (CC), patients heterozygous for the minor allele (CG) and patients without the minor allele (GG). Black bar, median plasma concentration; white box, interquartile range.

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 controls with liver disease, 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. (2005) showed that transgenic mice overexpressing S100A4 displayed increased thickness of the internal and external lamina of the pulmonary arteries. 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 remodelling (Yanagisawa et al. 2002).

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 haemolysis or inflammatory changes could also raise the plasma S100A4 levels (Flatmark et al. 2004, Klingelhöfer et al. 2007).

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 probably led to increased plasma S100A4 levels, which have been shown to be a robust marker of hepatic fibrosis (Rygiel et al. 2008). 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 multicentre cohort of PPHTN patients, this 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 end-stage liver disease (Colle et al. 2003). 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 with liver disease, 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 non-specifically increase plasma S100A4 or the high circulating levels of S100A4 in patients with advanced liver disease could facilitate the development of PPHTN.

Acknowledgements

We thank Charles A. Powell, May Huang, John O'Connor and the other staff at the Irving Institute for Clinical and Translational Research for their assistance. The study was funded by NIH grants DK064103, DK065958, RR00645, RR00585, RR00046, RR00032, RR024156 and HL67771.

***Declaration of interest*:** S.M.K. has received lecture fees, consultancy fees and/or other support from Actelion, Pfizer, Gilead, Lilly, Novartis, INO Therapeutics, United Therapeutics, Gerson Lehrman and Clinical Advisors. M.J.K. has provided consultation regarding possible clinical trials concerning portopulmonary hypertension for Gilead, United Therapeutics and Actelion. The other authors have not declared a potential conflict of interest.

The pulmonary vascular complications of liver disease study group

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References

Colle IO, Moreau R, Godinho E, Belghiti J, Ettori F, Cohen-Solal A, Mal H, Bernuau J, Marty J, Lebrec D, Valla D, Durand F. 2003. Diagnosis of portopulmonary hypertension in candidates for liver transplantation: a prospective study. *Hepatology* 37:401–409.

Flatmark K, Maelandsmo GM, Mikalsen SO, Nustad K, Varaas T, Rasmussen H, Meling GI, Fodstad Ø, Paus E. 2004. Immunofluorometric assay for the metastasis-related protein S100A4: release of S100A4 from normal blood cells prohibits the use of S100A4 as a tumor marker in plasma and serum. *Tumour Biol* 25:31–40.

Garrett SC, Varney KM, Weber DJ, Bresnick AR. 2006. S100A4, a mediator of metastasis. *J Biol Chem* 281:677–680.

Greenway S, van Suylen RJ, Du Marchie Sarvaas G, Kwan E, Ambartsumian N, Lukanidin E, Rabinovitch M. 2004. S100A4/Mts1 produces murine pulmonary artery changes resembling plexogenic arteriopathy and is increased in human plexogenic arteriopathy. *Am J Pathol* 164:253–262.

Hintze J. NCSS and PASS 2005. Kaysville, Utah: Number Cruncher Statistical Systems, 2004.

Kawut SM, Krowka MJ, Trotter JF, Roberts KE, Benza RL, Badesch DB, Taichman DB, Horn EM, Zacks S, Kaplowitz N, Brown RS Jr, Fallon MB for the Pulmonary Vascular Complications of Liver Disease Study Group. 2008. Clinical risk factors for portopulmonary hypertension. *Hepatology* 48:196–203.

Kawut SM, Taichman DB, Ahya VN, Kaplan S, Archer-Chicko CL, Kimmel SE, Palevsky HI. 2005. Hemodynamics and survival of patients with portopulmonary hypertension. *Liver Transpl* 11:1107–1111.

Klingelhöfer J, Senolt L, Baslund B, Nielsen GH, Skibshøj I, Pavelka K, Neidhart M, Gay S, Ambartsumian N, Hansen BS, Petersen J, Lukanidin E, Grigorian M. 2007. Up-regulation of metastasis-promoting S100A4 (Mts-1) in rheumatoid arthritis: putative involvement in the pathogenesis of rheumatoid arthritis. *Arthritis Rheum* 56:779–789.

Krowka MJ, Mandell MS, Ramsay MA, Kawut SM, Fallon MB, Manzarbeitia C, Pardo M Jr, Marotta P, Uemoto S, Stoffel MP, Benson JT. 2004. Hepatopulmonary syndrome and portopulmonary hypertension: a report of the multicenter liver transplant database. *Liver Transpl* 10:174–182.

Krowka MJ, Swanson KL, Frantz RP, McGoon MD, Wiesner RH. 2006. Portopulmonary hypertension: results from a 10-year screening algorithm. *Hepatology* 44:1502–1510.

Lawson WE, Polosukhin VV, Zoia O, Stathopoulos GT, Han W, Plieth D, Loyd JE, Neilson EG, Blackwell TS. 2005. Characterization of fibroblast-specific protein 1 in pulmonary fibrosis. *Am J Respir Crit Care Med* 171:899–907.

Merklinger SL, Wagner RA, Spiekerkoetter E, Hinek A, Knutsen RH, Kabir MG, Desai K, Hacker S, Wang L, Cann GM, Ambartsumian NS, Lukanidin E, Bernstein D, Husain M, Mecham RP, Starcher B, Yanagisawa H, Rabinovitch M. 2005. Increased fibulin-5 and elastin in S100A4/Mts1 mice with pulmonary hypertension. *Circ Res* 97:596–604.

Roberts KE, Fallon MB, Krowka MJ, Brown RS, Trotter JF, Peter I, Tighiouart H, Knowles JA, Rabinowitz D, Benza RL, Badesch DB, Taichman DB, Horn EM, Zacks S, Kaplowitz N, Kawut SM for the PVCLD Study Group. 2009. Genetic risk factors for portopulmonary hypertension in patients with advanced liver disease. *Am J Resp Crit Care Med* (in press).

Rygiel KA, Robertson H, Marshall HL, Pekalski M, Zhao L, Booth TA, Jones DE, Burt AD, Kirby JA. 2008. Epithelial–mesenchymal transition contributes to portal tract fibrogenesis during human chronic liver disease. *Lab Invest* 88:112–123.

Simonneau G, Galiè N, Rubin LJ, Langleben D, Seeger W, Domenighetti G, Gibbs S, Lebrec D, Speich R, Beghetti M, Rich S, Fishman A. 2004. Clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 43(12 Suppl. S):5S–12S.

Sussman N, Kaza V, Barshes N, Stribling R, Goss J, O'Mahony C, Zhang E, Vierling J, Frost A. 2006. Successful liver transplantation following medical management of portopulmonary hypertension: a single-center series. *Am J Transplant* 6:2177–2182.

Yanagisawa H, Davis EC, Starcher BC, Ouchi T, Yanagisawa M, Richardson JA, Olson EN. 2002. Fibulin-5 is an elastin-binding protein essential for elastic fibre development in vivo. *Nature* 415:168–171.