ORIGINAL ARTICLE

Apolipoprotein A-Ib as a biomarker of focal segmental glomerulosclerosis recurrence after kidney transplantation: diagnostic performance and assessment of its prognostic value – a multi-centre cohort study

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SUMMARY

Recurrence of idiopathic focal segmental glomerulosclerosis (FSGS) is a serious complication after kidney transplantation. FSGS relapse is suspected by a sudden increase in proteinuria but there is not an accurate noninvasive diagnostic tool to confirm this entity or to detect patients at risk. We aimed to validate the diagnostic performance of ApoA-Ib to detect FSGS relapses by measuring urinary ApoA-Ib in a retrospective cohort of 61 kidney transplanted patients (37 FSGS and 24 non-FSGS). In addition, to assess the ApoA-Ib predictive ability, ApoA-Ib was measured periodically in a prospective cohort of 13 idiopathic FSGS patients who were followed during 1 year after transplantation. ApoA-Ib had a sensitivity of 93.3% and a specificity of 90.9% to diagnose FSGS relapses, with a high negative predictive value (95.2%), confirming our previous results. In the prospective cohort, ApoA-Ib predated the recurrence in four of five episodes observed. In the nonrelapsing group ($n = 9$), ApoA-Ib was negative in 37 of 38 samples. ApoA-Ib has the potential to be a good diagnostic biomarker of FSGS relapses, providing a confident criterion to exclude false positives even in the presence of high proteinuria. It has also the potential to detect patients at risk of relapse, even before transplantation.

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Key words
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Introduction

Primary or idiopathic focal segmental glomerulosclerosis (FSGS) is a clinical entity associated with idiopathic nephrotic syndrome (INS) of unknown cause that does not respond to steroid therapy in about half of the cases [1] and frequently progress to end-stage renal failure [2–5]. FSGS is a confusing term since it is a nonspecific histological pattern observed in other unrelated conditions such as genetic alterations of podocyte proteins, reflux nephropathy, obesity or AIDS, which are collectively termed as secondary FSGS [6]. On the other hand, idiopathic FSGS does not seem to be a unique disease itself, but rather the common feature of several underlying etiologies [2,7]. Idiopathic and secondary FSGS can be differentiated because the latter does not display the complete effacement of podocyte foot process characteristic of idiopathic FSGS [8].The diagnosis of idiopathic FSGS is based on the presence of proteinuria, a histological pattern of FSGS in a kidney biopsy, and excluding secondary and genetic causes [9]. A major problem of idiopathic FSGS is its recurrence after kidney transplantation in about 30–50% of the patients [10,11]. It often leads to graft failure, requiring a new transplant which has an even higher probability for FSGS recurrence itself [12]. Several therapeutic approaches have been proposed to prevent and treat relapses, with variable success [13–16]. Early post-transplant relapse is mainly clinically detected by the sudden increase in proteinuria. The role of the biopsy at this stage is limited because the typical histological lesions are not present and appear later, at least 1 month after the increase in proteinuria, making diagnostic confirmation and early treatment difficult [17–19].

Regarding the requirement of a histological pattern of segmental glomeruloesclerosis for the diagnosis of idiopathic FSGS, Maas et aI. [18.] proposed that idiopathic FSGS and minimal change disease (MCD), another INS without glomerular sclerosis and generally responsive to steroids, are two manifestations of the same disease, sharing the effacement of podocyte foot process. MCD would correspond to an early, lesion-free stage of the disease, while FSGS would correspond to a later phase with evident segmental glomerulusclerosis lesions. This concept would cast doubts on the requirement of segmental glomerulosclerosis lesions in kidney biopsies to diagnose idiopathic FSGS.

Given the difficulties in the diagnosis of this entity, the identification of biomarkers that would allow an early and specific diagnosis of FSGS relapses after transplantation could benefit this population from preventive and early therapeutic interventions. In our previous work [20] we described a modified form of Apolipoprotein A-I (ApoA-Ib) that appeared in the urine of relapsing FSGS patients, and was absent in other forms of proteinuria.

In the present work, we intend to confirm our findings in an independent cohort, as well as investigate the presence of ApoA-Ib in FSGS-unrelated transplanted patients with variable levels of proteinuria. We also aim to explore the possibility that the appearance of ApoA-Ib predates the FSGS relapses, thus allowing the detection of patients at risk of recurrence after transplantation.

Patients and methods

Clinical design

Between January 2013 and December 2015 urine samples were obtained from kidney transplanted patients treated in nephrology departments of 16 major Spanish hospitals. The samples were collected for two different purposes:

ApoA-Ib in the diagnosis of FSGS recurrence

We evaluated a retrospective cohort to assess the value of ApoA-Ib as biomarker of FSGS recurrence. To this end, we collected urine samples of three groups of patients. The first group consisted of patients with FSGS recurrence (FSGS-R) defined as the appearance of proteinuria ≥1.5 g/24 h and histological confirmation. The urine sample was obtained at the time of recurrence before starting treatment with plasmapheresis. The second group consisted of patients with FSGS without recurrence during at least one year after transplantation (proteinuria <1.5 g/24 h) or patients with FSGS with proteinuria >1.5 g because of a different histological diagnosis from FSGS (FSGS-NR). Finally, the third group consisted of patients without FSGS as the primary disease with variable degrees of proteinuria (No-FSGS).

ApoA-Ib as a predictor of FSGS recurrence after transplantation

To evaluate whether APOA-Ib predicts the risk of relapse we performed a prospective cohort study. Patients in the transplant waiting list with primary or idiopathic biopsy-proven FSGS were invited to participate and the ones that received a kidney transplant were followed after transplantation. ApoA-Ib was determined at 1 week, 1 month,

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3 months and 1 year or whenever a biopsy was indicated because of proteinuria ≥ 1.5 g/d. An episode of recurrence was defined by the increase in proteinuria and the histological findings in the kidney biopsy.

This study was approved by the Vall d'Hebron Hospital Ethics Committee (PR-IR 103/2008), and informed consent was obtained from all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki and Declaration of Istanbul.

Sample collection and determination of ApoA-Ib

A 15-ml amount of urine was collected and, after a centrifugation at 1500 *g* to eliminate the urinary sediment, it was stored at −80 °C. Urinary samples can be stored ultrafrozen for months without changes in the results, as demonstrated by repeated measures of the same samples used as controls for more than 6 years in our laboratory. Hematic urine samples were excluded, because we have detected false positive values when macroscopic hematuria is observed.

All urinary samples were concentrated by ultrafiltration and ApoA-Ib was determined by western blot as described previously [20]. Briefly, proteins were separated in SDS-PAGE gels with tris-glycine-SDS buffer, transferred to PVDF membranes, probed with anti-ApoA-I (rabbit polyclonal PAB8546; Abnova, Jhongli, Taiwan, plus HRP-antirabbit secondary antibody P0448; Dako, Glostrup, Denmark). Proteins were detected by chemiluminescence (Luminata Forte, Millipore and detector LAS3000, Fujifilm, Tokyo).

Statistical analyses

Comparisons between groups were carried out using ANOVA test followed by Tukey's multiple comparisons test. When variance homogeneity requirements were not met, the Kruskal–Wallis and Dunn's multiple comparisons test were applied. Chi-squared test was used to compare qualitative variables. Clopper-Pearson confidence intervals were used for specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV). To evaluate whether urine ApoA-Ib is independently associated with FSGS relapse, a bias-corrected logistic regression analysis was performed considering urine ApoA-Ib as dependent variable and serum creatinine and daily proteinuria as independent variables. Statistical analyses were done with commercially available software (GRAPHPAD PRISM 6.0; Graphpad Software, La Jolla, CA, USA) except for the bias-corrected logistic regression that was done with R software, Version 3.4.0 (R Core Team, 2017).

Results

ApoA-Ib in the diagnosis of FSGS recurrence

A total of 37 patients with end stage renal disease because of biopsy proven FSGS (Table 1) were transplanted and a relapse of FSGS occurred in 15 patients (FSGS-R). The recurrence incidence in our retrospective cohort was 40%. Most of them relapsed during the first year (n = 11), and three patients before the third month after transplantation. Only four patients relapsed after one year post transplantation. Clinical data of these patients are summarized in Table S1. The remaining 22 transplanted patients with biopsy proven FSGS did not relapse and constituted the FSGR-NR group. In six out of these 22 patients a renal biopsy was done because of proteinuria ≥1.5 g/24 h to rule out FSGS recurrence. Histological diagnoses were humoral rejection (n = 3), interstitial fibrosis and tubular atrophy (n = 2) and thrombotic microangiopathy (n = 1) (Table S2). The No-FSGS group consisted of 24 transplanted patients (Table 1) with primary disease diagnosis different from FSGS (Table S3). In this group, 11 patients showed stable renal function without significant proteinuria and 13 patients had proteinuria >1.5 g/24 h because of relapse of the primary disease (n = 5) or IFTA (n = 8) (Table S3).

The Fig. 1 depicts the proportion of patients with positive ApoA-1b in each group. In the FSGS-R group 14 of 15 (93%) were positive for apoA-Ib whereas only two of 22 (9%) in FSGS-NR group, and three of 24 (12%) in No-FSGS group were tested positive for ApoA-Ib (P value <0.001) (Fig. 1). As detailed in Table 2, ApoA-Ib sensitivity and specificity to detect FSGS recurrence were 93.3% and 90.9% to discriminate nonrelapsing FSGS patients or 93.3% and 87.5% to discriminate transplanted patients without FSGS as the primary disease.

ApoA-Ib is independent of proteinuria and serum creatinine

Proteinuria and serum creatinine individual values of the patients included in each group have been plotted in Fig. 2. There were no statistical differences in the degree of proteinuria and serum creatinine between FSGS-R and No-FSGS group (Table 1), despite most of

the patients tested positive (14 of 15) for Apo A-Ib in the FSGS-R group and negative (21 out of 24) in the No-FSGS group (Fig. 2). Furthermore, we performed a bias-corrected logistic regression to test whether ApoA-Ib was independent of proteinuria or serum creatinine levels. This analysis was performed only considering FSGS-R and No-FSGS groups. ApoA-Ib positivity was set as the dependent variable and serum creatinine and daily proteinuria as independent variables. The logistic regression showed that ApoA-Ib was independent of both proteinuria (Odds ratio (OR) 1.11, 95% Confidence Interval (CI) (0.95–1.41)) and serum creatinine levels (Odds ratio (OR) 1.09, 95% Confidence Interval (CI) (0.76–1.60)).

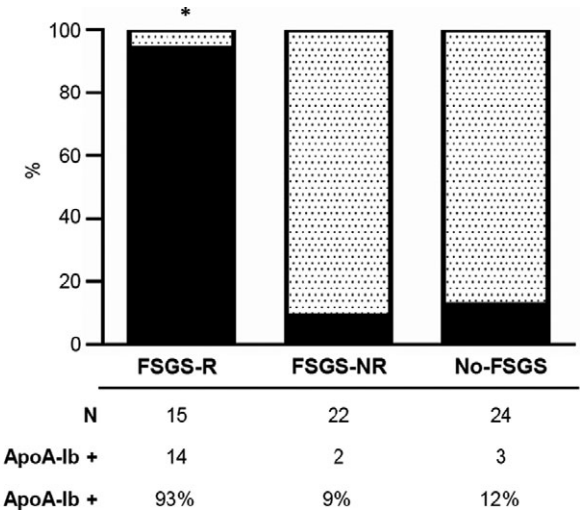

Figure 1 Urinary ApoA-Ib as biomarker for FSGS post-transplant relapse. Statistical comparison of the urinary ApoA-Ib distribution in the studied groups (FSGS-R, FSGS-NR and CTL). ApoA-Ib positive patients are shown in black, ApoA-Ib negative in dotted white. ApoA-Ib in FSGS-R is significantly higher than in the other groups (Chi-squared test, (*) *P* value <0.001).

ApoA-Ib as a predictor of FSGS recurrence after transplantation

A total of 33 patients diagnosed with FSGS included in the waiting list were evaluated. There were 25 patients on hemodialysis and eight predialysis patients. In four patients on dialysis a urine sample for ApoA-Ib analysis could not be obtained (Table S4). In this group of not transplanted FSGS patients ApoA-Ib tested positive in 11 of 29 patients with urine output (37.9%).

Seventeen of these patients received a kidney transplant and 13 were followed during the first year (Fig. 3). There were four relapsing patients and in three of them ApoA-Ib was positive before the recurrence. Nine patients did not show recurrence, eight were always negative while one presented a single ApoA-Ib positive value at 1 month after transplantation (Table 3). As detailed in Table 3, patients FSGS-33 and FSGS-9 had ApoA-Ib in urine before transplantation. ApoA-Ib was detected in all the set of samples of patient FSGS-33, who had an immediate recurrence of FSGS with no remission. Patient FSGS-9 was ApoA-Ib positive before and after transplantation, before relapse diagnosis, and became negative after remission (ApoA-Ib measurements for these patients are shown in Fig. S1). Patient FSGS-16 was anuric before transplantation and ApoA-Ib could not be determined at this point. Interestingly, this patient presented two relapses; one immediately after transplantation and a second relapse one year after the transplantation. In both

Table 1. Clinical data of the patients.

	Transplanted patients		
	FSGS-R FSGS relapsing *n* = 15	FSGS-NR FSGS nonrelapsing *n* = 22	No-FSGS FSGS-unrelated *n* = 24
Men/women	8/7	14/8	16/8
Age, years	45.1 ± 4.4	45.1 ± 3.2	46.0 ± 3.9
Creatinine, mg/dl*	2.44 (1.15; 3.73)	1.47 (1.25; 1.70)*	2.52 (1.80; 3.23)
Proteinuria, g/24 h*	5.18 (2.15; 8.21)	0.83 (0.35; 1.30)*	3.24 (1.85; 4.23)
Albumin, g/dl	3.35 (2.96; 3.74)	4.16 (3.90; 4.42)	3.57 (3.20; 3.94)
Cholesterol, mg/dl	216.7 (171.9; 261.6)	184.2 (173.8; 194.6)	211.8 (188.0; 235.6)
Triglycerides, mg/dl	168.2 (128.2; 208.2)	137.4 (114.8; 160.0)	208.6 (109.6; 307.7)

Data are expressed as the mean (95% CI of the mean). All NS except *($P < 0.001$). FSGS-NR versus FSGS-R and No-FSGS (Kruskal–Wallis test and Dunn's multiple comparisons test).

Table 2. Performance of ApoA-Ib to detect FSGS post-transplant relapses.

Detection of FSGS relapses	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
FSGS-R versus FSGS-NR	93.3 (68.1; 99.8)	90.9 (70.8; 98.8)	87.5 (61.5; 98.4)	95.2 (76.2; 99.8)
FSGS-R versus No-FSGS	93.3 (68.1; 99.8)	87.5 (67.6; 97.3)	82.4 (56.6; 96.2)	95.5 (77.1; 98.8)
FSGS-R versus (FSGS-NR+No-FSGS)	93.3 (68.1; 99.8)	89.1 (77.3; 96.5)	73.7 (48.8; 90.8)	97.6 (87.9; 99.9)

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in percentage with Clopper-Pearson 95% confidence intervals.

episodes ApoA-Ib could be detected before the onset of proteinuria. This patient responded to plasmapheresis after the second relapse and ApoA-Ib turned negative (data not shown). The only discordant result was obtained for the patient FSGS-12, who was diagnosed of FSGS relapse 2 months after transplantation, but none of the previous urine samples was positive for ApoA-Ib.

Discussion

The diagnosis of FSGS relapses after kidney transplantation relies on a clinical feature and a histological feature: the sudden increase in proteinuria usually to a nephrotic range and the presence of segmental glomerulosclerosis. However, there are several conditions than can produce both findings [21]. The increase in proteinuria can be originated by other causes, or can be neglected if the increase is not high enough to reach the nephrotic range. The histological signs of FSGS do not develop until at least one month after the onset of the proteinuria [17], and even when the glomerulosclerosis is present it can be missed by sampling error [19]. Thus, the diagnosis of FSGS relapse has a high positive predictive value when the classical features coincide, i.e., severe proteinuria and segmental glomerulosclerosis lesions in the biopsy, but their absence do not exclude the diagnosis.

Value of ApoA-Ib as a biomarker of FSGS relapses

In our previous study [20] we described the presence of ApoA-Ib, a modified form of Apolipoprotein A-I, in urine of patients with post-transplant recurrence of FSGS. The method we used to determine ApoA-Ib only requires a sample of fresh urine, which is stable for months when stored adequately. To validate the association of ApoA-Ib with post-transplant FSGS recurrence, we have studied a prospective cohort of 61 new patients, which included 37 FSGS. In these series we have confirmed that ApoA-Ib appears in FSGS relapses and not only distinguishes from FSGS not relapsing

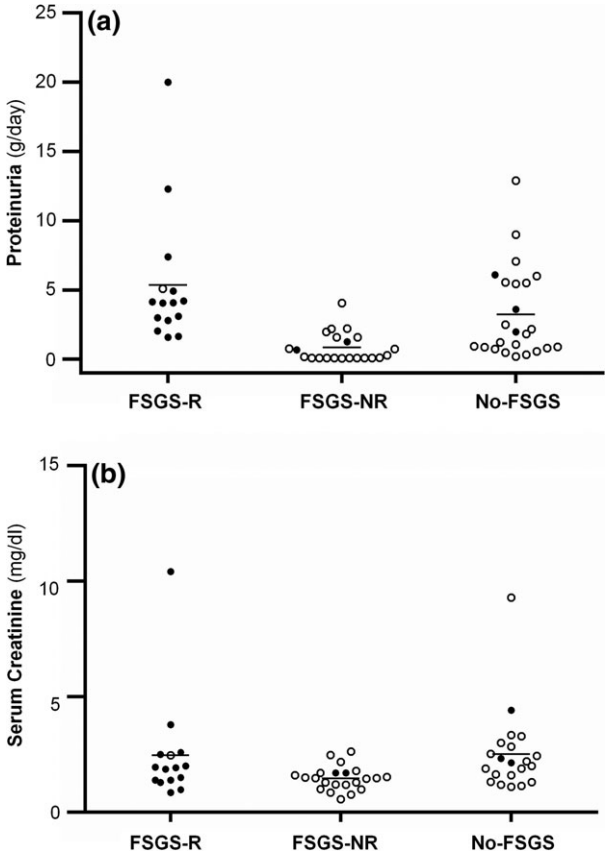

Figure 2 Urinary ApoA-Ib is not related to proteinuria or serum creatinine levels. Individual values of proteinuria (a) and serum creatinine levels (b) of the patients included in each group. ApoA-Ib positive patients are represented as black dots and ApoA-Ib negative patients as white dots. The mean of each group is shown as a line. ApoA-1b is positive in urine of FSGS-R patients in a broad range of proteinuria (a) and serum creatinine levels (b) and in patients FSGS-NR and CTL in the same ranges of proteinuria (a) or serum creatinine (b) ApoA-Ib is negative.

patients as expected, but also from kidney transplanted patients with underlying pathologies not related to FSGS with a good specificity (87.5%) and excellent sensitivity (93.3%). The presence of ApoA-Ib is related to FSGS relapses after transplantation, but not to other etiologies of allograft damage presenting with proteinuria

(Fig. 1). This feature can be of special interest in early biopsies after the recurrence, when the biopsy may not be informative. In these cases, the determination of ApoA-Ib in urine could be helpful in the diagnosis of FSGS relapses because it is rarely detected in cases of FSGS-unrelated post-transplantation proteinuria (12.5%). Moreover, the high negative predictive value of ApoA-Ib (95.2%) indicates that the marker is specially suited to preclude a diagnosis of FSGS when ApoA-Ib is negative, thus complementing the high positive predictive value of the kidney biopsy. These data demonstrate that ApoA-Ib can be used as a biomarker to distinguish post-transplant recurrence of FSGS from other unrelated diseases with similar clinical characteristics.

Among the 24 patients included in the No-FSGS group, 11 patients that presented recurrent glomerulonephritis in their native kidney were analyzed (seven membranoprolipherative gromerulonephritis (MPGN), two membranous gromerulonephritis (MN) and two Ig A nephropathy) (see Table S3). In this last subgroup we detected three false positive patients for ApoA-Ib: two MPGN and one MN. Even so, only two of these patients showed recurrence of the primary disease after kidney transplantation. Our results strongly suggest that urinary ApoA-Ib is specifically related to FSGS recurrence (see Table 2), but to rule out an association of ApoA-Ib with other glomerular recurrent diseases a larger cohort of this kind of patients should be further investigated.

Value of ApoA-Ib in the prediction of FSGS relapses

The prognostic value of ApoA-Ib is complex to demonstrate because requires following an important number of patients from the diagnosis of idiopathic FSGS up to more than one year after transplantation. The fact that some of the patients in the waiting list were anuric, as

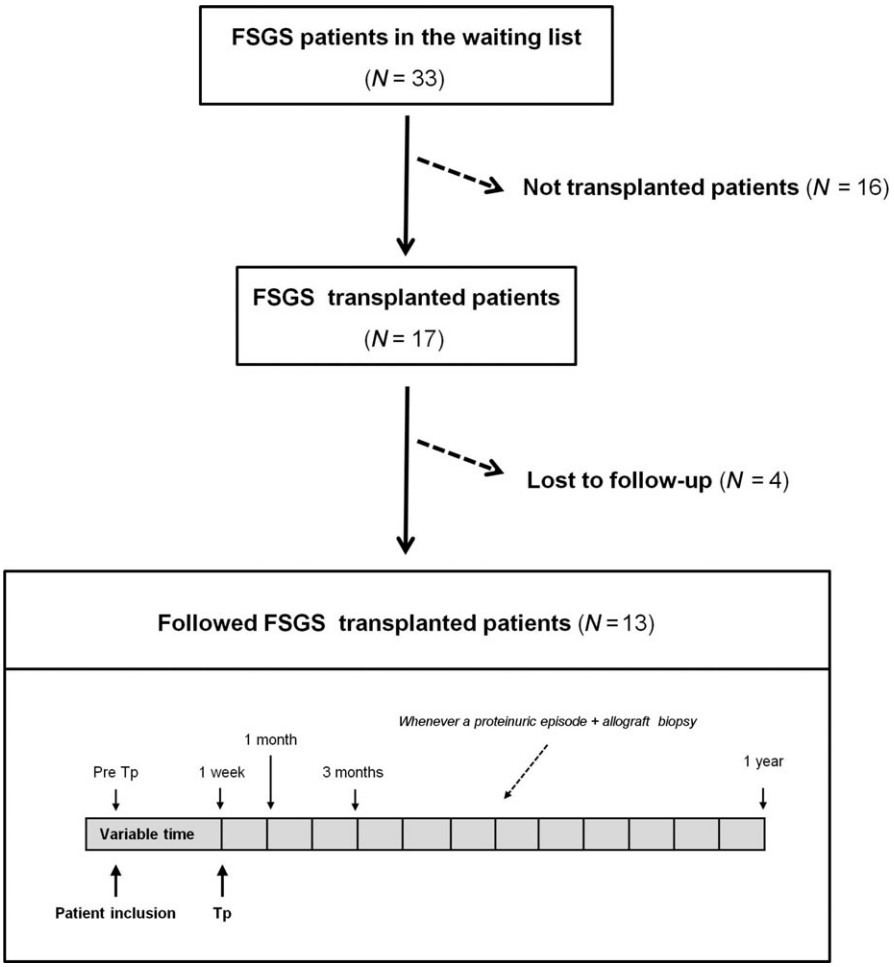

Figure 3 Experimental design and sampling schedule to test the predictive value of ApoA-Ib for post-transplant relapses of FSGS.

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Table 3. Urinary ApoA-1b, proteinuria and serum creatinine corresponding to the samples collected during 1 year after transplantation of the 13 followed idiopathic FSGS patients.

			Post-Trasplantation							
	Patient	Pretrasplantation	1 week		1 month		3 months	1 Year		
Relapse	**FSGS 33**									
	ApoA-Ib	Positive	Positive	Relapse	Positive		Positive	Positive†		
	Proteinuria (g/24 h)	37	6.8		14.3		12.6	12.8		
	Creatinine (mg/dl)	5.44	3.47		1.74		2.01	2.08		
	FSGS 9									
	ApoA-Ib	Positive	Positive	Relapse	Negative†		Negative†	Negative		
	Proteinuria (g/24 h)	2.9	4.07		2.6		0.8	0.49		
	Creatinine (mg/dl)	9.95	1.5		0.99		1.49	1.05		
	FSGS 16									
	ApoA-Ib	Anuria	Positive	Relapse	Positive		Negative	Positive	Relapse	
	Proteinuria (g/24 h)		1.5		4.4		1.39	1.6		
	Creatinine (mg/dl)		2.43		1.77		1.89	1.4		
	FSGS 12									
	ApoA-Ib	Negative	–		Negative	Relapse	Negative	Negative		
	Proteinuria (g/24 h)	0.54			2.6		1.5	1.7		
	Creatinine (mg/dl)	2.07			0.94		1.23	1.3		
No relapse	**FSGS 6**									
	ApoA-Ib	Anuria	n.d.*		Negative		–	Negative		
	Proteinuria (g/24 h)		0.25		<0.1			<0.1		
	Creatinine (mg/dl)		3.4		1.1			1		
	FSGS 13									
	ApoA-Ib	Negative	Negative		Negative		Negative	Negative		
	Proteinuria (g/24 h)	1.5	0.69		1.79		0.1	1.6		
	Creatinine (mg/dl)	5.6	1.73		1.49		1.31	1.6		
	FSGS 15									
	ApoA-Ib	Negative	Negative		Negative		Negative	Negative		
	Proteinuria (g/24 h)	0.4	0.4		0.16		0.24	15.28‡		
	Creatinine (mg/dl)	1.1	1.1		0.96		0.93	1.56		
	FSGS 20									
	ApoA-Ib	Negative	Negative		Negative		Negative	Negative		
	Proteinuria (g/24 h)	n.d.	0.3		<0.1		<0.1	<0.1		
	Creatinine (mg/dl)	5.25	1.85		1.6		1.37	1.7		
	FSGS 21									
	ApoA-Ib	Negative	Negative		Negative		Negative	Negative		
	Proteinuria (g/24 h)	n.d.	<0.1		<0.1		<0.1	<0.1		
	Creatinine (mg/dl)	8	2.77		1.07		1.47	0.99		
	FSGS 23									
	ApoA-Ib	Anuria	Negative		Negative		Negative	Negative		
	Proteinuria (g/l)		0.3		<0.1		0.49	<0.1		
	Creatinine (mg/dl)		3.06		1.57		1.67	1.48		
	FSGS 24									
	ApoA-Ib	Negative	–		Positive		Negative	Negative		
	Proteinuria (g/24 h)	0.49			0.31		<0.1	<0.1		
	Creatinine (mg/dl)	9.5			2.1		2	1.8		

Table 3. Continued.

Patient	Pretrasplantation	Post-Trasplantation			
		1 week	1 month	3 months	1 Year
FSGS 25					
ApoA-Ib	Anuria	Negative	–	Negative	Negative
Proteinuria (g/24 h)		1.64		<0.1	0.1
Creatinine (mg/dl)		4.87		1.3	1.3
FSGS 28					
ApoA-Ib	Negative	Negative	Negative	Negative	Negative
Proteinuria (g/24 h)	n.d.	1.64	1.05	0.4	0.77
Creatinine (mg/dl)	4.56	5.45	3.2	2.75	2.18

–: Missing sample; n.d.: not determined.

*Macroscopic hematuria.

†Plasmapheresis.

‡Chronic humoral rejection.

expected, diminished even more the number of samples that potentially could be collected. Bearing in mind these difficulties, we designed a study to test whether ApoA-Ib could be detected before the relapses, considering both possibilities that ApoA-Ib appeared before or after the transplantation, but always before the recurrence of the disease.

Four of 13 followed patients had a FSGS recurrence during the first year after transplantation and ApoA-Ib predicted it correctly in three of them. ApoA-Ib was positive before transplantation in two patients out of the four patients in whom FSGS recurred (Table 3, FSGS-33 and FSGS-9).

The episode of recurrence in patient FSGS-33 did not respond to treatment (plasmapheresis) and did not remit during the period of study, and in all urine samples collected during one year tested positive for ApoA-Ib (Table 3 and Fig. S1). On the other hand, patient FSGS-9 responded to plasmapheresis after recurrence and ApoA-Ib turned negative when the disease remitted (Table 3 and Fig. S1). Patient FSGS-16, despite being anuric before transplantation, was positive for ApoA-Ib previously to an immediate post-transplantation relapse, that remitted spontaneously and, again, ApoA-Ib turned negative (Table 3). These observations, particularly in patient FSGS16, who relapsed twice, suggest that the time lag between appearance of ApoA-Ib and the relapse itself may be short. Moreover, the remaining patients who presented no recurrence were negative for ApoA-Ib in all their samples, not only during the first year after transplantation but also in the subsequent years,. There was only one exception inpatient FSGS-24 who tested positive in one sample (Table 3). As expected, none of the nonrelapsing patients showed high levels of proteinuria during one year after transplantation, except for FSGS-15 at the end of the follow-up period. In this case a recurrence of FSGS was ruled out by a biopsy that showed clear signs of chronic humoral rejection (Table 3). As ApoA-Ib in urine is not dependent of proteinuria levels (Fig. 2a) the ApoA-Ib results observed in these patients can be directly attributed to FSGS relapses. Additionally, we have observed that the proportion of FSGS patients in the waiting list positive for ApoA-Ib in urine (37.9%) was similar to the mean incidence of FSGS relapses after transplantation, that is estimated to be around 30–50% [10,11], supporting the predictive value of ApoA-Ib.

The principal limitation of our study is the number of patients included in both cohorts. Idiopathic FSGS is a disease with a low incidence ranging from 0.2 to 1.8/

100.000 per year, depending on the cohort [22,23], and recurrence occurs in 30–50% of the patients once transplanted [10,11]. Therefore, similar studies on FSGS recurrence have been done also in small cohorts [24–26]. Although we are aware that the number of patients included is not enough to reach a definitive conclusion, our data strongly suggest that ApoA-Ib is useful to assess the risk of recurrence at an early stage, even before transplantation.

The results presented in this work confirm that ApoA-Ib is a specific biomarker for FSGS recurrence and seems to have a promising predictive value. ApoA-Ib determination in idiopathic FSGS patients could be helpful not only to diagnose with certainty FSGS relapses but also to stratify patients at risk of relapse. Currently, there are no clear criteria to start preventive treatment in FSGS relapses, and deciding which patient would benefit from preventive treatment can be difficult in the clinical setting. The early detection of ApoA-Ib would provide evidence for a better preventive intervention on these patients.

Finally, the fact that idiopathic FSGS may be caused by a still unknown plasmatic factor [22,27–29], and that ApoA-Ib is specifically related to FSGS relapses and disappears upon remission of FSGS recurrence both spontaneously or in response to treatment, allow to suggest a potential role for this abnormal form of Apolipoprotein A-I in the pathogenesis of FSGS, either in a direct or an indirect way. This line of investigation is currently being pursued in our laboratory in order to elucidate the implication of ApoA-Ib on FSGS pathophysiology, together with the characterization of the protein.

Conclusion

We have demonstrated in an independent cohort that ApoA-Ib can be used as a biomarker for post-transplant recurrence of FSGS. The absence of ApoA-Ib in urine allows ruling out a diagnosis of FSGS relapse with a high accuracy, even in the presence of severe proteinuria. It also complements the classical diagnostic criteria based on the histological findings in a kidney biopsy, particularly in early cases in which the classical lesions have not been yet developed. Moreover, it has the major advantage of being a noninvasive biomarker, allowing easy and repeated measurements with a simple qualitative result. Finally, our data clearly point that ApoA-Ib could be useful to detect patients at risk of FSGS recurrence after transplantation.

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Authorship

JLH, CC, FJM, DS: Conception and design. NPG, CJC, JS, CC, JLH: Analysis and interpretation of the data and drafting the article. LG, FGR, CJ, SZ, JP, RL, AA, AF, IB, AM, DH, ARB, AF, LJ, MC, AM: Data collection and revising the article.

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Conflict of interest

None declared.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Example of ApoA-Ib measurement in serial urine samples from relapsing patients.

Table S1. Proteinuria individual values, time post-transplantation, ApoA-Ib in relapsing FSGS patients.

Table S2. Proteinuria individual values, time post-transplantation, ApoA-Ib in nonrelapsing FSGS patients and histological diagnosis.

Table S3. Etiology of FSGS-unrelated transplanted patients included in the study, individual values of ApoA-Ib and proteinuria and underlying reason of control after kidney transplantation.

Table S4. Individual values of proteinuria, hemodialysis state and urinary ApoA-Ib of the nontransplanted FSGS patients.

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