Supplemental Material – Table of Contents

Supplemental Table 1. Published studies on incidence of recurrent FSGS since 1990 including > 20 subjects.

Year	Population	Total n	Incidence	95% CI	Multiple Tx	Analysis	Ethnicity	Risk factors
1990 [4]	Children + Adults	59	22%	11-33	Yes	Univariable	Unknown	Younger age onset FSGS, Mesangial prominence
1990 [5]	Children + Adults	25	48%	28-68	Yes	Univariable	Unknown	
1991 [6]	Children	40	15%	4-26	Yes	Univariable	Mixed	Younger age onset FSGS
1991 [7]	Adults	47	30%	17-43	Yes	Univariable	Unknown	Shorter duration of kidney disease
1992 [9]	Children	132	21%	14-27	Yes	Univariable	Mixed	Rapid progression to ESKD
1992 [11]	Children + Adults	78	25%	22-42	Yes	Univariable	Mixed	Rapid progression to ESKD, white ethnicity
1996 [10]	Children + Adults	114	20%	13-28	Yes	Multivariable	Mixed	Nephrectomy native kidneys
1999 [8]	Children	29	52%	34-70	No	Univariable	Unknown	Older age onset FSGS, rapid progression to ESKD*
2001 [12]	Adults	27	48%	29-67	No	Multivariable	Asian	Higher donor age
2006 [13]	Adults	35	34%	19-50	Yes	Univariable	Unknown	Higher donor age
2008 [16]	Children	37	43%	27-59	No	Univariable	Mixed	
2009 [14]	Children + Adults	30	47%	29-65	No	Univariable	Mixed	Younger age at transplant, lower number HLA mismatches, living-related donor, higher pre-transplant peak proteinuria, treatment with Cyclosporine before transplant.
2009 [17]	Adults	22	23%	5-40	No	Univariable	Unknown	Younger age onset FSGS, rapid progression to ESKD, living donor, nephrectomy native kidneys, higher pre-transplant peak proteinuria, female gender
2010 [15]	Adults	52	23%	12-34	Yes	Univariable	Unknown	Younger age onset FSGS, male gender, duration of dialysis
2010 [18]	Adults	66	23%	13-33	No	Univariable	Mixed	
2010 [19]	Children + Adults	77	55%	43-66	No	Univariable	Unknown	
2011 [22]	Children + Adults	131	17%	10-23	Yes	Univariable	Mixed	Younger age at transplant
2012 [23]	Children + Adults	107	28%	20-37	No	Univariable	Asian	
2013 [20]	Children + Adults	66	42%	31-54	No	Multivariable	Mainly white	Lower serum albumin at diagnosis
2018 [21]	Children	158	41%	33-48	No	Multivariable	Mixed	Histology of minimal change disease compared to FSGS

CI, confidence interval; FSGS, focal segmental glomerulosclerosis; Tx, transplants.

*The abstract of this article has different outcomes than reported in the text. Risk factors are selected from the main text of the article.

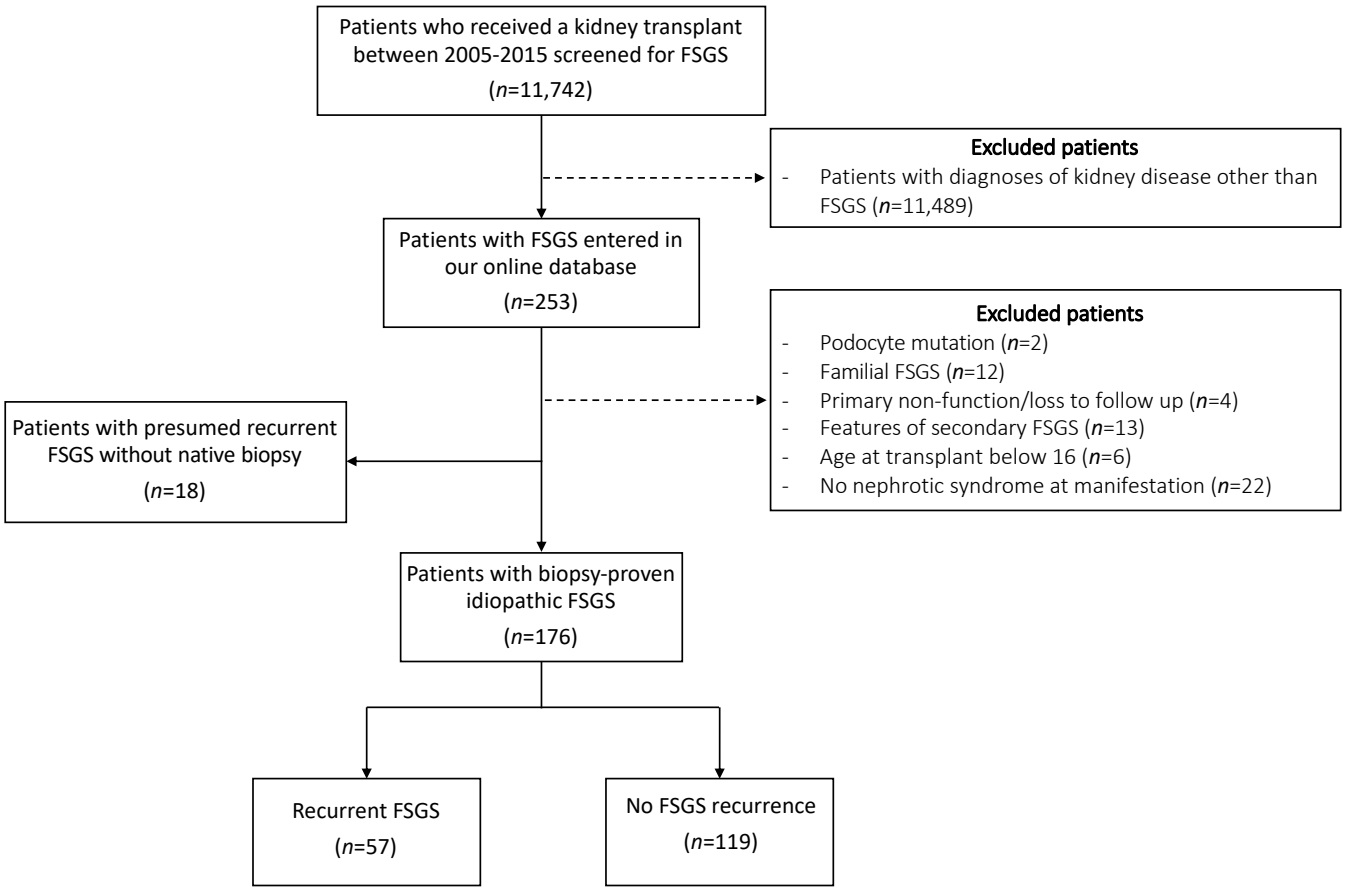
Supplemental Table 2. Missing data in predictors for patients without and with recurrent FSGS

Variable	No recurrence (*n*=119)	Recurrence (*n*=57)	*P*-value
Age at diagnosis	2 (2)	2 (4)	0.596
White race	10 (8)	9 (16)	0.193
BMI	0 (0)	1 (2)	0.324
Time on dialysis	0 (0)	2 (4)	0.104
Nephrectomy of native kidneys	1 (1)	0 (0)	1.000
Age donor	11 (9)	3 (5)	0.553
HLA-mismatch	7 (6)	3 (5)	1.000
DSA at transplant	10 (8)	5 (9)	1.000
Use of induction	3 (3)	0 (0)	0.552
Immunosuppressive regimen	1 (1)	0 (0)	1.000
Continent	0 (0)	0 (0)	NA
Type of transplant	0 (0)	0 (0)	NA
Prophylactic plasmapheresis	0 (0)	0 (0)	NA
Total missing values (% of all data)	45 (3)	25 (3)	

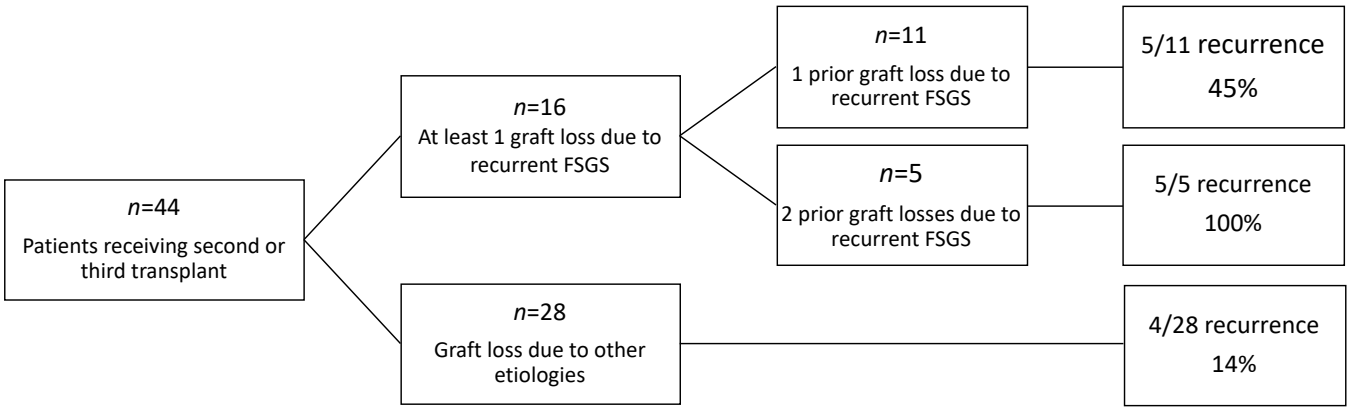
Supplemental Table 3. Univariable logistic regression for any response to treatment.

Variable	No response to treatment (*n*=26)	Any response to treatment (*n*=35)	Odds ratio (95% CI)	*P*-value
Recurrence within 6 months	20 (77)	28 (80)	0.83 (0.24-2.86)	0.772
Age at transplantation, *per year*	41 (28-56)	33 (27-44)	0.97 (0.93-1.01)	0.104
Female gender	8 (31)	18 (51)	2.38 (0.82-6.91)	0.110
White race	15 (68)	19 (59)	0.68 (0.22-2.14)	0.511
Geographical location				
USA	7 (27)	13 (37)	Ref	Ref
Brazil	10 (38)	16 (46)	0.86 (0.26-2.89)	0.810
Europe	9 (35)	6 (17)	0.36 (0.09-1.43)	0.146

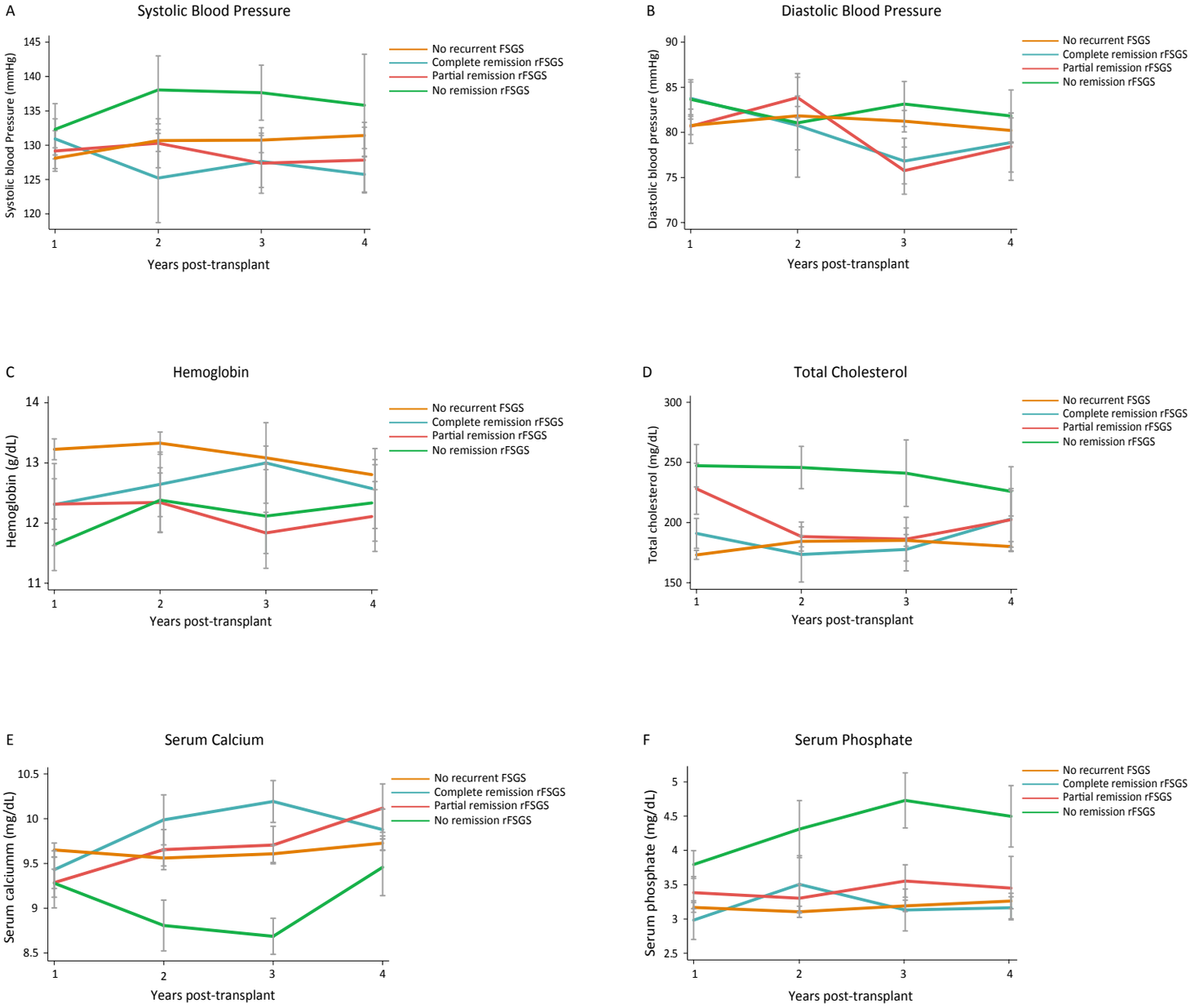
Supplemental Figure 1. Flowchart of the study population.



Supplemental Figure 2. Prior graft loss due to recurrent FSGS and risk of recurrence in subsequent allografts.



Supplemental Figure 3. Clinical outcomes post-transplantation in patients with a functioning allograft. Comparison of levels of **(A)** systolic- and **(B)** diastolic blood pressure, **(C)** Hemoglobin, **(D)** cholesterol, **(E)** calcium and **(F)** phosphate levels in patients without recurrence with patients with recurrent FSGS stratified by their response to treatment of recurrent FSGS. Error bars represent standard errors. FSGS, focal segmental glomerulosclerosis; rFSGS, recurrent focal segmental glomerulosclerosis.



Supplemental Methods

Collected data

Collected data comprised patient demographics, medical history, information on native biopsy, transplantation characteristics, immunosuppressive regimen and data collected at annual post-transplant visits including clinical parameters, rejection, FSGS recurrences and other complications. Patients were censored at the first of graft loss, patient death, loss to follow-up, or in January 2019.

Patient selection (continued)

In two participating centers, Hospital do Rim and Cajuru University Hospital, both in Brazil, native kidney biopsies were not available for all patients. Therefore, these two centers were excluded from the primary analysis on incidence and predictive factors of FSGS. However, most of their patients with a pretransplant kidney disease history highly suspicious of FSGS (*e.g.*, sudden onset of nephrotic syndrome) and who had nephrotic-range proteinuria post-transplant, underwent an allograft biopsy for diagnosis. If this post-transplant biopsy showed FSGS features, the patient was included in analyses on treatment efficacy of recurrent FSGS.

Predictor selection

Informed by prior literature and clinical practice, [4–22,24–27,34] we selected and collected data on the following potential predictors of recurrent FSGS: age at disease onset, race/ethnicity, BMI at time of transplantation, time to ESKD, dialysis vintage, nephrectomy of native kidneys, type of donor (living vs. deceased), age of the donor, HLA mismatch, donor-specific antibodies (DSA) prior to transplant, use of induction therapy, pretransplant prophylactic plasmapheresis and initial immunosuppressive regimen. To account for the different geographical regions in which the patients were followed-up, continent of residence was also added as a predictor.

Data storage

All data was stored in an online database that was designed for TANGO-study, using REDCap™ (Research Electronic Data Capture): a secure, HIPAA-compliant web-based application [48] hosted by the Partners HealthCare Research Computing, Enterprise Research Infrastructure and Services (ERIS) group. Investigators received access to the secured website to enter patient data online, and/or to access data only from their center. Upon downloading of the dataset, event dates were date-shifted to further de-identify the dataset to ensure confidentiality of participants.

Definitions

Recurrent FSGS was detected by nephrotic-range proteinuria post-transplant and was confirmed by kidney biopsy showing either FSGS lesions by light microscopy or diffuse foot process effacement by electron microscopy. In 9 cases, FSGS was not biopsy-confirmed but was immediately treated with plasmapheresis or immunosuppression, due to the high clinical suspicion for recurrent FSGS. These patients were considered to have recurrent FSGS. Three patients developed proteinuria while being on mTOR inhibitors, and proteinuria decreased after switching to another immunosuppressant agent (tacrolimus). These three patients were not considered to have recurrent FSGS.

Treatment response of recurrent FSGS was divided into complete, partial, or no remission. Complete remission was defined as a reduction of proteinuria below 0.3 g/24h (or 0.3 g/g of urinary creatinine) with stable eGFR (*e.g.*, maximum decline of 15%). Partial remission was defined as a reduction of proteinuria below 2.0 g/24h (or 2.0 g/g), or a reduction of proteinuria of at least 50% of the highest value to a level below 3.5g/24h (or 3.5 g/g), both with stable eGFR. eGFR was estimated by the Modification of Diet in Renal Disease (MDRD) Study equation.[49]

Delayed graft function was defined as the need for hemodialysis within the first week post-transplant. Acute antibody- or cellular-mediated rejection was recorded if confirmed on kidney biopsy by the pathologist of the corresponding center. Borderline rejection was not considered acute rejection. New onset diabetes was defined as a new and persistent elevation of blood glucose levels post-transplantation requiring glucose-lowering medication.

Potential sources of Bias

We implemented the following strategies to avoid potential sources of bias: Centers were instructed to chronologically add patients according to their date of transplant, to avoid selection bias towards patients who had a recurrence. We recorded detailed medical histories to be able to make a proper evaluation for secondary causes of FSGS. Review of histories and biopsies of patients included in our database was done in a blinded fashion for the primary outcome of post-transplant FSGS recurrence. Each case of post-transplant recurrence was reviewed and when questions were raised (*e.g.*, no treatment was given, recurrence occurred very late after transplant), clarification was asked from the specific center to verify it was a true recurrence. Clarification of centers on specific patients was also asked when yearly post-transplant proteinuria values suddenly increased and persisted without clear cause, but no recurrent FSGS was recorded. Analyses to graft failure and complications post-transplant were corrected for the most important confounders known from literature. The multi-center setup of this study over multiple continents was done to make sure many ethnical groups were present to avoid population bias. Unfortunately, some ethnical groups (especially patients with an Asian background) were still underrepresented. An analysis plan with clearly defined outcome and predictors (selected from literature) was made before the start of data-analysis and was followed throughout the analysis of data.

Imputation

Little's missing completely at random (MCAR) test was performed on all predictors and outcome to investigate randomness of missing data, and resulted in a significant outcome (p=0.0068), which implies that the pattern of missing data was not completely at random. However, detailed analysis of missing data showed a low frequency of missing data (overall 3%) and Fisher's exact test showed no difference per predictor between recurrence groups (Supplemental Table 2), after which we proceeded with imputation. STATA's multiple imputation by chained equations (MICE) procedure was used to impute missing categorical, ordinal, normal continuous and non-normal continuous variables by logistic regression, ordinal regression, linear regression and predictive mean matching, respectively. Imputations were made using all predefined predictors, including recurrence and graft failure. For each missing value, 100 values were imputed. In case of perfect prediction, augmentation was performed to avoid bias in imputations. Imputations were graphically assessed on outliers and variances and coefficients were checked on agreement.

Sensitivity analyses

Because missing data was imputed by MICE, we performed a sensitivity analysis to determine the impact of the chosen method for handling missing data. Complete case analysis on the final model (geographic location of center, age at diagnosis FSGS, white race, BMI and nephrectomy of native kidneys) resulted in an analysis of 151 patients. Similar to the imputed model, significant p-values for all variables were observed, except for nephrectomy of native kidneys (95% CI: 0.62-5.85). An explanation for the difference in outcome between complete case and imputed model was found in the pattern of missing data: in 25 patients who were excluded from the complete case multivariable analysis due to missing in one of the parameters, 5 patients had a nephrectomy of native kidneys (in the total cohort, 12

patients had a nephrectomy). Therefore, analysis of imputed data for this variable seems more reliable than complete case multivariable analysis.

In our analysis plan, we had planned to perform a sensitivity analysis on the multivariable model, by performing the final multivariable model on the patients of each possible combination of two continents, to see whether significance of variables would hold. When we executed this method, most significant parameters held, except nephrectomy when patients from Europe or the United States were removed, and race when patients of the USA were removed. The explanation for this can be found in the numbers of nephrectomies done, as there were none performed in Brazil. When removing United States or Europe, the total number of patients with a nephrectomy got too low (5 or 7) for analysis. Regarding race, most non-white patients were included in the USA, while only one non-white person was included from Europe. Therefore, when USA patients were removed from the total sample size, significance for white race did not hold.

Ethical considerations

The overall protocol of TANGO-study was submitted and approved by the ethical committee of the Partners Human Research Committee (PHRC) at the Brigham and Women's hospital in Boston (protocol number: 2015P000993), and at each participating center. In one participating center, the University Medical Center Groningen, ethical approval was waived by the Medical Ethics review Board (METc UMCG). All protocols are in accordance with International Conference on Harmonization Good Clinical Practice Guidelines and the Declaration of Helsinki. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the 'Declaration of Istanbul on Organ Trafficking and Transplant Tourism'.