JAK-STAT signaling is activated in the kidney and peripheral blood cells of patients with focal segmental glomerulosclerosis

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Focal segmental glomerular sclerosis (FSGS) is a devastating disease with limited treatment options and poor prognosis. Activated JAK-STAT signaling has been implicated in other kidney diseases. Since new technologies allow us to better evaluate changes in systemic and renal JAK-STAT activity as it relates to kidney function, we examined this in 106 patients with biopsy-proven FSGS compared to 47 healthy control individuals. Peripheral immune function was assessed in peripheral blood mononuclear cells by phosphoflow studies before and after cytokine stimulation. Kidney JAK-STAT activity was measured by immunofluorescence and by transcriptomics. A STAT1 activity score was calculated by evaluating message status of downstream targets of pSTAT 1. Peripheral blood mononuclear cells were found to be upregulated in terms of pSTAT production at baseline in FSGS and to have limited reserve to respond to various cytokines. Increased staining for components of the JAK-STAT system in FSGS by microscopy was found. Furthermore, we found transcriptomic evidence for activation of JAK-STAT that increased pSTAT 1 and pSTAT 3 in glomerular and tubulointerstitial sections of the kidney. Some of these changes were associated with the likelihood of remission of proteinuria and progression of disease. JAK-STAT signaling is altered in patients with FSGS as compared to healthy controls with activated peripheral immune cells, increased message in the kidney and increased activated proteins in the kidney. Thus, our findings support immune activation in this disease and point to the JAK-STAT pathway as a potential target for treatment of FSGS.

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Focal segmental glomerulosclerosis (FSGS) is a group of diseases sharing a common glomerular lesion of segmental glomerular sclerosis and hyalinosis.[1] It accounts for approximately 20% of cases of nephrotic syndrome in children and nearly 40% in adults.[2] Over the last 30 years, the prevalence of FSGS in the United States (US) has increased from 14% to 20%[3] among primary glomerulonephritis, and it is the leading glomerular disorder causing end-stage renal disease (ESRD)[4] in the US.

Traditionally, idiopathic FSGS, a podocytopathy,[5] is thought to be caused, at least in part, by a disorder of circulating lymphocytes or T cell dysfunction, potentially through the release of an unidentified "permeability factor,"[6] which is toxic to the glomerular ultrafiltration barrier.[7] Many attempts have been made to elucidate the nature of this factor, although it remains elusive.[8] Knowledge about disorders of circulating lymphocytes in FSGS is even more limited due to limitations in research technology. Human peripheral blood mononuclear cells exist in a heterogeneous pool comprising 70% T cells, 10% B cells, and another 10% monocytes. In 2000, utilizing 3 channels as the most advanced technology for flow cytometry at that time, Stachowski *et al.*[9] found the activity of T-helper-1 (Th1) and T-helper-2 (Th2) cells and specific lymphocyte subsets, namely CD45RA+CD4+ ("naive" helper T cells, suppressor-inducer), CD45RA+CD8+ ("naive" cytotoxic T cells, cytotoxic-effector), and CD45RO+CD4+ ("memory" helper T cells) were predictive of steroid sensitivity in FSGS. The work suggested a strategy to further study the function of peripheral blood mononuclear cells (PBMCs) as a disease pathway and biomarker of FSGS.

Phospho-flow is a more recently developed research strategy based on flow cytometry. It can simultaneously distinguish cell subsets in PBMCs and quantify the phosphorylation levels of intracellular signaling proteins such as signal transducer and activator of transcription (STAT).[10] Present phospho-flow platforms allow us to precisely separate PBMCs into subsets as CD4+, CD4+CD45RA+, CD4+CD45RA–, CD8+, CD8+CD45RA+, CD8+CD45RA–, natural killer cells, B lymphocytes, and monocytes. Janus kinase (JAK)-STAT is a major pathway that responds to and transduces inflammatory signals from cells through extracellular ligands such as cytokines and chemokines.[11]

Recently, compelling preliminary data in kidney cell–specific over-expression and knockout transgenic animal

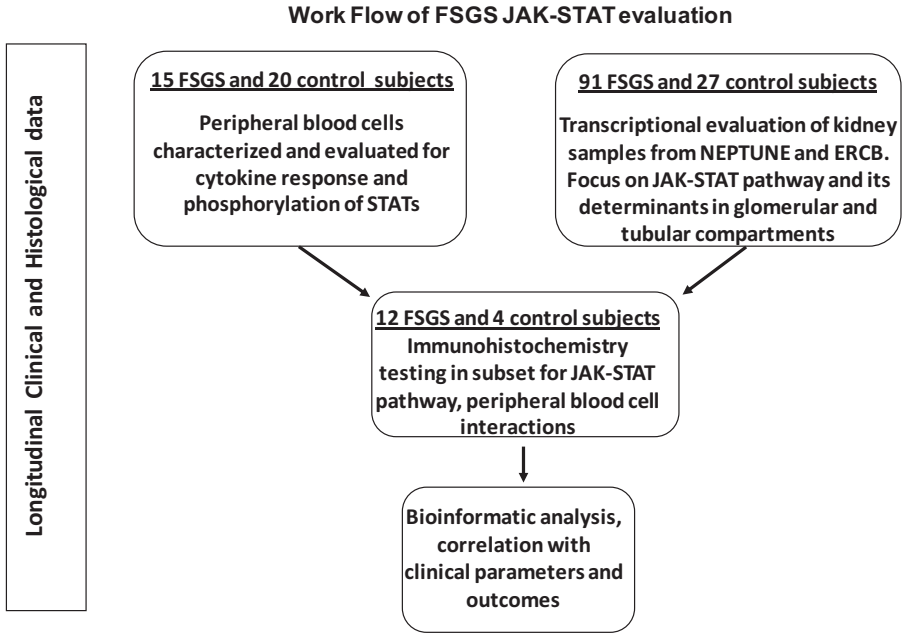

Figure 1 | Work flow of focal segmental glomerular sclerosis (FSGS) Janus kinase–signal transducer and activator of transcription (JAK-STAT) evaluation. ERCB, European Renal cDNA Bank.

models, as well as in genetic profile analysis in human samples, have revealed that JAK-STAT is extensively activated in diabetic nephropathy, autosomal dominant polycystic kidney disease, HIV-associated nephropathy, acute kidney injury, and obstructive uropathy,[12] in which the JAK2-STAT3 pathway is the most involved. This has already resulted in active trials of medications that inhibit the JAK-STAT pathway in diabetic nephropathy and kidney transplantation.[13,14] Thus far, in primary glomerular disease, only 1 study analyzing the genomic profile of PBMCs from 3 Ig A nephropathy patients highlighted the role of STAT1 in the exacerbation of gross hematuria.[15] It thus seemed prudent to explore JAK-STAT in mediating the immune response systemically or locally in the kidney as a major role in the pathogenesis of idiopathic FSGS.[16]

In this study, we, for the first time, examined the phosphorylation of STAT1, STAT3, and STAT5 in each of these subsets with or without *in vitro* cytokine stimulation. We also explored JAK-STAT expression and content in the kidney and associated it with disease activity and outcomes (Figure 1).

Table 1 | Baseline characteristics of 15 patients with FSGS with PBMCs assayed in phospho-flow

Age at biopsy (yr)	37 (24–61)
Female	9 (60%)
African American	2 (13%)
Asian	4 (26%)
White	4 (26%)
Hispanic	5 (33%)
Sampling time after biopsy (ds)	351 (63–2884)
eGFR at PBMC sampling (ml/min per 1.73 m^2)	50 (26–133)
Urine protein excretion at PBMC sampling (g/g)	2.8 (0.5–6.1)

eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; PBMC, peripheral blood mononuclear cell.

METHODS

Patients

In the initial part of this study, 15 adult patients with biopsy-proven idiopathic FSGS were evaluated and provided their blood for analysis of PBMCs (Figure 1). All subjects provided informed consent. Healthy controls ($n = 20$) matched for age (42.9 ± 14.56 years) and gender (10 men and 10 women) were also studied. Patients were not on any active immunotherapy for at least the prior 6 months. Their general characteristics are listed in Table 1. Kidney tissue from 9 FSGS patients were studied by immunohistochemistry for JAK1, JAK2, STAT3, pSTAT3, and pSTAT1. Their baseline characteristics are also summarized in Table 2. Five patients had both PBMCs assayed and biopsy slides stained. A separate group of 72 patients (Table 3) with biopsy-proven FSGS from NEPTUNE were evaluated. NEPTUNE, part of the National Institutes of Health (NIH) Rare Disease Clinical Research Network (RDCRN), is a multicenter prospective cohort study enrolling patients with proteinuric glomerular disease and performing comprehensive clinical and molecular phenotyping. Patients with secondary glomerular disease (such as diabetic kidney disease, lupus nephritis, and amyloidosis) were excluded. Comparable healthy tissue was obtained from living transplant donors for comparison with NEPTUNE ($n = 6$) FSGS biopsy samples (transcriptional group, with added patients from European Renal cDNA Bank; Figure 1). Biopsy material from both cohorts was microdissected into glomerular and tubulointerstitial compartments

Table 2 | Baseline characteristics of 9 patients with FSGS with kidneys stained for JAK1, JAK2, STAT3, pSTAT3, and pSTAT1

Age at biopsy (yr)	56 (28–69)
Female	4 (44%)
White Hispanic	4 (44%)
White non-Hispanic	5 (56%)
eGFR (ml/min per 1.73 m^2)	76 (24–124)
Urine protein excretion (g/g)	3.5 (1.7–7.2)

eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis.