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CLINICAL RESEARCH

Implementation and Feasibility of Clinical Genome Sequencing Embedded Into the Outpatient Nephrology Care for Patients With Proteinuric Kidney Disease

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Introduction: The diagnosis and management of proteinuric kidney diseases such as focal segmental glomerulosclerosis (FSGS) are challenging. Genetics holds the promise to improve clinical decision making for these diseases; however, it is often performed too late to enable timely clinical action and it is not implemented within routine outpatient nephrology visits.

Methods: We sought to test the implementation and feasibility of clinical rapid genome sequencing (GS) in guiding decision making in patients with proteinuric kidney disease in real-time and embedded in the outpatient nephrology setting.

Results: We enrolled 10 children or young adults with biopsy-proven FSGS (9 cases) or minimal change disease (1 case). The mean age at enrollment was 16.2 years (range 2–30). The workflow did not require referral to external genetics clinics but was conducted entirely during the nephrology standard-of-care appointments. The total turn-around-time from enrollment to return-of-results and clinical decision averaged 21.8 days (12.4 for GS), which is well within a time frame that allows clinically relevant treatment decisions. A monogenic or APOL1-related form of kidney disease was diagnosed in 5 of 10 patients. The genetic findings resulted in a rectified diagnosis in 6 patients. Both positive and negative GS findings determined a change in pharmacological treatment. In 3 patients, the results were instrumental for transplant evaluation, donor selection, and the immunosuppressive treatment. All patients and families received genetic counseling.

Conclusion: Clinical GS is feasible and can be implemented in real-time in the outpatient care to help guiding clinical management. Additional studies are needed to confirm the cost-effectiveness and broader utility of clinical GS across the phenotypic and demographic spectrum of kidney diseases.

Kidney Int Rep (2023) **8,** 1638–1647; https://doi.org/10.1016/j.ekir.2023.05.021
KEYWORDS: feasibility; focal segmental glomerulosclerosis; genetic diagnosis; genome sequencing; implementation; proteinuria

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Received 19 March 2023; revised 1 May 2023; accepted 22 May 2023; published online 26 May 2023

FSGS is among the most severe forms of nephrotic syndrome (NS), particularly in children,[1,2] characterized by high morbidity, poor response to therapy, and high rate of progression to kidney failure requiring dialysis or transplantation.[3] FSGS describes a histologic lesion secondary to the injury and depletion of podocytes, the glomerular epithelial cells responsible for maintaining the kidney filtration

barrier. FSGS can be primary or idiopathic, genetic, or secondary to diverse conditions (hypertension, obesity, drugs, viruses, and others).[4] The histopathological definition of FSGS does not reflect the underlying pathogenic insult, thus preventing adequate assessment of prognosis and individualized treatment. Minimal change disease can present with significant clinical and prognostic overlap with FSGS, especially in adults, though it is more frequently responsive to steroids and immunosuppressives.[5]

In younger children, NS is usually treated empirically with high-dose steroids because up to 80% to 90% of cases of NS are steroid-sensitive (i.e., steroid-sensitive NS).[6] This empirical approach subjects the patient to prolonged immunosuppressive therapy in the absence of a specific diagnosis, which proves unnecessary and harmful in up to 20% of children.[7] Furthermore, the prevalence of steroid-sensitive NS decreases with age, and often teenagers presenting with NS undergo kidney biopsy before initiation of steroid treatment, which is the current practice in adults. In general, when a full course of steroids fails to induce remission of NS (i.e., steroid-resistant NS), a kidney biopsy is indicated, with FSGS representing the most common histopathologic finding on biopsy.[2,3] In steroid-resistant NS, therapeutic options include additional immunosuppressive drugs or conservative therapy while preparing for kidney transplantation. Consequently, the majority of children and young adults with NS may be exposed to prolonged, toxic, and potentially ineffective immunosuppressive therapy, irrespective of their underlying kidney pathology and its specific etiology.

Previous studies have demonstrated a monogenic cause in approximately 20% to 30% of patients with steroid-resistant NS who are below the age of 18 years.[8,9] Genetic forms of NS are usually resistant to immunosuppressive treatment and do not recur following kidney transplantation.[10,11] In addition to the avoidance of unwarranted immunosuppression and prognostic value, genetic analysis has other benefits. These include allowing targeted therapy, for example by administration of oral supplements of coenzyme Q10 in patients with coenzyme Q biosynthesis deficiency-associated steroid-resistant NS.[12,13] Finally, genetic testing can identify variants predisposing to extrarenal conditions such as hearing defects in COL4A-associated disease, that might be subclinical or manifesting later in life, and that might be amenable to targeted treatment.[14,15] Therefore, genetic testing at the time of diagnosis and/or accompanying the outpatient work-up and management of glomerular diseases in real-time during standard-of-care nephrology visits holds the promise to optimize care by avoiding unnecessary and toxic immunosuppression; identifying possible etiologic treatment; and informing and improving transplant evaluation, treatment plan, and family counseling.

Next-generation sequencing technologies such as exome and GS are increasingly used in the molecular diagnosis of kidney diseases, with recent studies showing a diagnostic Mendelian cause in approximately 10% of adults with chronic kidney disease.[16,17] Nevertheless, these studies have been conducted in the research setting on established and retrospective cohorts of patients and disjointed from the regular outpatient care, thus reducing the direct impact of genetic findings at the level of individual patient care.[18–20] Studies aimed at investigating the feasibility and the real-time diagnostic and clinical utility of next-generation sequencing have been mostly directed to critically ill infants admitted to neonatal and pediatric intensive care units, where the pretest probability of a genetic etiology is very high.[21,22] These studies have proven instrumental in clinical decision making but they were performed in a highly controlled clinical setting where long inpatient length-of-stay, close monitoring, and deep phenotyping make a rapid clinical genetic screening more feasible than in an older population of children and young adults who are in an outpatient setting.[21] Therefore, the implementation of clinical-grade rapid genetic testing in children and young adults, especially those in the outpatient setting presents greater challenges and its implementation and feasibility have yet to be tested.

Here we present the results of a pilot study designed to apply a multidisciplinary approach to outpatients diagnosed with proteinuric kidney disease, in which Clinical Laboratory Improvement Amendments–certified rapid GS is conducted to guide therapy and real-time clinical management in the outpatient setting and conducted during standard-of-care nephrology visits.

METHODS

Please refer to the Supplementary Appendix for the complete methods, consent process, and study timeline.

Clinical Protocol and Procedures

Patients were enrolled in the Division of Nephrology at Columbia University Irving Medical Center/NewYork-Presbyterian Hospital, the Division of Pediatric Nephrology at Columbia University Irving Medical Center/NewYork-Presbyterian Morgan Stanley Children's Hospital, and the Division of Pediatric Nephrology at University of California, Los Angeles.

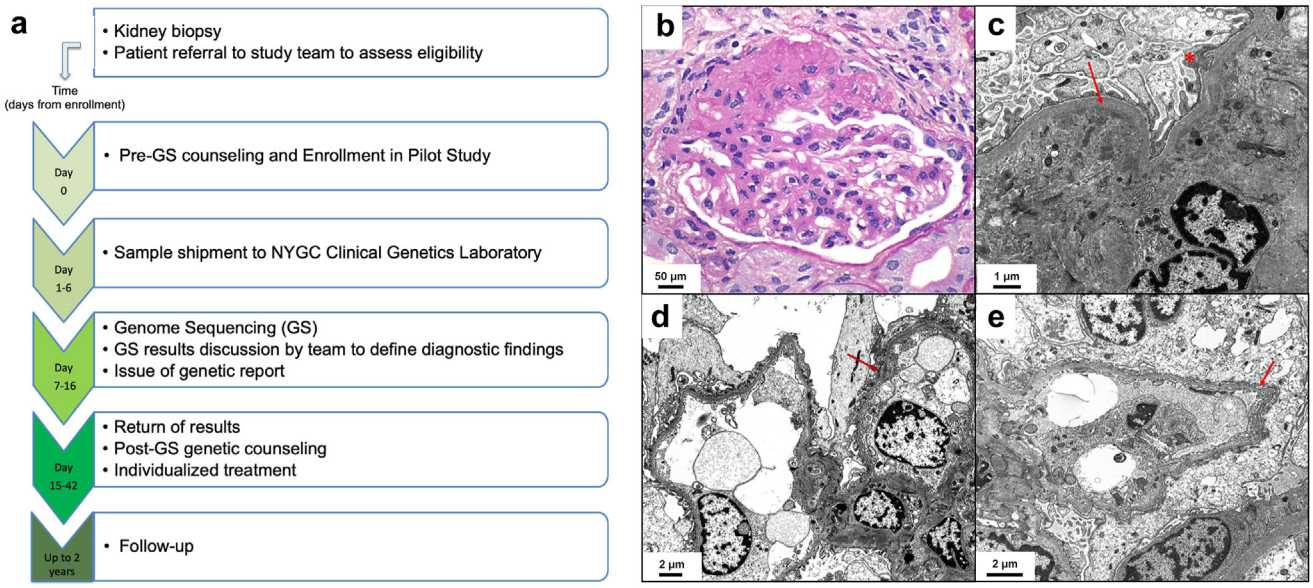

Figure 1. Panel (a) Study protocol timeline. Potential patients with a kidney biopsy showing pathological changes consistent with focal segmental glomerulosclerosis (FSGS) were referred ahead of time to the study team by the caring nephrologist or pathologist who first read the biopsy to assess patient eligibility based on study criteria, pathologic features, and meaningful/impact of genome sequencing (GS) results in clinical management.

Day 0. Following pre-GS counseling with the explanation of the study to the patient and family by the investigational team, patients and/or guardian(s) signed both the Columbia University IRB-approved study consent/assent form and a clinical genetic test consent form. One 5 ml EDTA tube of blood for DNA analysis was collected from the patient and from parents if available.

Day 1–6. The blood sample(s) were shipped to the New York Genome Center Clinical Genetics Laboratory for DNA analysis.

Day 7–16. After completion of GS and variant analysis the investigative team discussed the results and issued the genetic report.

Day 15–42. The referring physician scheduled a multidisciplinary visit with the study investigators for return of results to the patient and/or to the parents. This included post-GS genetic counseling and genetic-driven individualized treatment.

Up to 2 years. Clinical follow-up based on standard-of-care and on genetic-driven decisions.

Panels (b–e). Representative pathologic findings in patients whose kidney biopsies were re-reviewed following results of genetic testing

Patient #2, panels (b and c): 12-year-old female with history of presumed "recurrent urinary tract infections," 2.1 grams of proteinuria and negative genetic testing. A representative glomerulus shows a lesion of segmental sclerosis with loss of overlying podocytes, disruption of Bowman's capsule, and prominent periglomerular inflammation. The adjacent nonsclerotic segments have mild mesangial hypercellularity with patent capillaries. (b; Periodic acid-Schiff, ×600). Immunofluorescence showed 1+ granular segmental to global mesangial staining for IgM (not shown). Ultrastructural examination revealed segmental mesangial expansion by increased mesangial cellularity and matrix containing mesangial electron dense deposits (arrow) with rare resorbed subepithelial deposits at the mesangial waist, leaving crater-like defects (asterisk), favoring a diagnosis of resolving infection-related glomerulonephritis (c; electron micrograph, ×8000).

Patient #10, panel (d): 29-year-old female with 1.4 grams of proteinuria, subsequently found to have a pathogenic variant in *COL4A5*, prompting rereview of her biopsy. Glomeruli were histologically unremarkable (not shown). Electron microscopy revealed mean glomerular basement membrane (GBM)thickness of 260 nm, with approximately 25% of the GBMs measuring <225 nm, consistent with segmental GBM thinning. There were also rare GBM textural irregularities consisting of splitting of the lamina densa (arrow). Podocytes displayed approximately 40% foot process effacement (d; electron micrograph, ×5,000).

Patient #4, panel (e): 8-year-old boy with 7.8 grams of proteinuria. Light microscopy revealed FSGS, NOS-type (not shown). Electron microscopy revealed 90% foot process effacement. The majority of capillary loops had GBMs of normal thickness, texture, and contour, with the exception of 2 capillaries with thinned GBM (measuring approximately 200 nm in thickness), raising the possibility of an underlying type IV collagen gene pathogenic variant. He was ultimately found to have a heterozygous *de novo* variant in *INF2* (e; electron micrograph, ×8,000). FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; GS, genome sequencing; NOS, not otherwise specified.

The workflow is described in Figure 1a and Supplementary Figure S1. Briefly, for patients with a biopsy showing pathological changes consistent with FSGS, the caring physician made a same-day referral to the study investigators to assess eligibility based on the potential for genetic-driven changes in clinical management. The renal pathologist (VDA) and the lead investigator (SS-C) reviewed the biopsy report and clinical data to determine eligibility for same-day enrollment. The inclusion criteria (see full description in the Supplementary Appendix) for enrollment in the study were based on high pretest probability of a genetic form of FSGS/NS as well as on high likelihood for changes in clinical care based on a positive or a negative genetic test result. On selection, the clinical coordinator immediately obtained the consent of the patient and/or family members. After obtaining informed consent from the patient and/or the guardian(s), blood

samples for clinical-level DNA analysis were obtained and sent for sequencing on the same-day nephrology visit. Finally, on the same day, we scheduled a follow-up visit for return of results and genetics-driven clinical decision within 3 weeks.

GS and Variant Interpretation

Following genomic DNA isolation from whole blood, GS was conducted to achieve a mean sequencing depth of 30×. Variant analysis was performed using the New York Genome Center's clinical pipeline, which follows the genomic analysis toolkit (GATK) best practices guidelines.[23] Genomic copy number alterations were identified using Canvas.[24] Structural variations were discovered using Manta.[25] We prioritized variants that occurred in a manually curated list of 678 nephropathy-associated genes (Supplementary Table S1 and S2)[16,17] before extending our analysis to Online Mendelian Inheritance in Man-annotated, Mendelian disease–associated genes.[26] Variant interpretation was guided by the patients' clinical presentation and phenotype. Reportable variants explaining the patient's disease and phenotype were classified using the American College of Medical Genetics and Genomics guidelines for DNA sequence variant interpretation.[27]

Return of Results and Clinical Decision Making

The return of results involved only the genes that, when mutated, explained the clinical diagnosis of our cases. These included Mendelian causes of NS/FSGS (*NPHS2, INF2*, etc.) or other kidney diseases that might phenocopy FSGS (*COL4A5, CLCN5*, etc.) but not incidental findings (Supplementary Tables S1 and S2).

After completion of GS and variant analysis, the investigative team and the referring physician conducted a multidisciplinary visit for return of results to the patient and genetic-driven clinical decision making. During this visit, the clinical genetic testing reports were entered in the patient's electronic health record.

Follow-Up

After the return of results, patients were followed by their treating nephrologists as per standard-of-care. The patient's electronic health record was reviewed by the study investigators at multiple follow-up timepoints up to 2 years from the day of return of results.

RESULTS

A detailed summary of the patient presentation, clinical questions that prompted genetic testing, genetic findings, and clinical course after the return of results is included in the Supplementary Appendix.

Baseline Clinical Characteristics

We enrolled 10 patients who met the inclusion criteria (Table 1). The mean age at enrollment was 16.2 years (range 2–30 years), 5 (50%) were female, 1 (10%) self-identified as Asian, and 6 (60%) as Hispanic/Latinx. Of the patients, 3 (30%) patients had a positive family history of kidney disease; 9 (90%) had biopsy-proven FSGS; and 1 showed features of minimal change glomerulopathy. Mean serum creatinine was 1 mg/dl (range 0.2–2 mg/dl). Five patients (cases 1, 3, 5, 6, and 8) had NS with urinary protein-to-creatinine ratio ranging from 6 to 15 mg/mg, edema, and low serum albumin. Three patients (cases 2, 4, and 9) had nephrotic-range proteinuria without NS. One patient (case #10) had subnephrotic range proteinuria (2.1 mg/mg) and 1 patient (case #7) was in remission on tacrolimus at the time of enrollment. For each patient, a specific clinical question was identified such as timely GS results would have helped in the following medical decisions (see Supplementary Appendix). Comorbidities included a history of hypertension (case #5), seizures (case # 7), and obesity (case #10).

Diagnostic Findings

The diagnosis of a monogenic form of kidney disease was made in 4 of 10 patients in the following genes:

Table 1. Clinical characteristics of the 10 patients enrolled in the pilot study

Pt.	Age	Sex	Race (self-reported)	Ethnicity (self-reported)	FHx	Histopathological Diagnosis	sCreat (mg/dl)	UPCR (mg/mg)	Current IS Therapy	Comorbidities
1	13	M	White	Non-Hispanic	No	FSGS, tip lesion	0.5	6	No	No
2	12	F	Unknown	Hispanic	No	FSGS, NOS	0.6	2.1	Yes	No
3	15	F	White	Non-Hispanic	Yes	FSGS, NOS	1.7	7	No	No
4	8	M	White	Hispanic	No	FSGS, NOS	0.4	7.8	Yes	No
5	26	M	White	Hispanic	Yes	FSGS, perihilar variant	1.1	7	Yes	Hypertension
6	22	M	White	Hispanic	No	FSGS, collapsing	1.3	9	Yes	No
7	5	M	White	Hispanic	No	FSGS, tip lesion	2	0.2	Yes	Seizures
8	2	F	White	Hispanic	No	FSGS, NOS (C1q variant)	0.2	15	Yes	No
9	30	F	Asian	Non-Hispanic	No	FSGS, perihilar variant	1.6	8.5	No	No
10	29	F	White	Non-Hispanic	Yes	Podocytopathy/minimal change disease	0.6	2.1	No	Obesity

F, female; *FHx*, Family history of kidney disease; *FSGS*, focal segmental glomerulosclerosis; *IS*, immunosuppressive therapy; *M*, male; *NOS*, "Not Otherwise specified" subtype of FSGS accordingly to the Columbia classification; *Pt.*, patient; *sCreat*, serum creatinine; *UPCR*, urine protein-to-creatinine ratio

Table 2. Diagnostic findings in 5 out of 10 cases

Pt.	Genetic Ancestry (%)	Gene	Protein Change	Zygosity	GnomAD MAF	Classification	Associated Genetic Condition (OMIM)
4	CSA (47) EUR (36) Other (17)	*INF2*	p.Leu77Pro	Het[a]	0	Likely pathogenic	FSGS-5 (613237)
5	AFR (34) EUR (48) Other (18)	*NPHS2*	p.Ala284Val p.Arg229Gln	Het Het	4×10^{-6} 0.03	Pathogenic VUS[b]	Nephrotic syndrome type 2 (600995) Nephrotic syndrome type 2 (600995)
6	AFR (29) CSA (6) EUR (47) Other (18)	*APOL1* *PAX2*	p.Asn404_Tyr405del p.His250Tyr	Hom Het	0.01 0	Risk factor (G2 allele) VUS	FSGS-4 (603743) FSGS-7 (616002)
9	SA (82) Other (18)	*COL4A4*	p.Gly341Asp	Het	0	Likely Pathogenic	Thin basement membrane disease (141200)
10	EUR (97) Other (3)	*COL4A5*	p.Arg1683Gln	Het	2×10^{-5}	Likely Pathogenic	X-linked Alport (301050)

AFR, African; *CSA*, Central/South American; *EUR*, European; *GnomAD*, Genome Aggregation Database; *Het*, heterozygous; *Hom*, homozygous; *MAF*, Medium Allele Frequency; *OMIM*, Online Mendelian Inheritance in Man; *Pt.*, patient; *SA*, South-Asian; *VUS*, variant of unknown significance
[a]Presumed *de novo* variant, tested by targeted Sanger sequencing in the parents without genome-wide paternity test.
[b]Although this *NPHS2* variant is classified as VUS as per the current American College of Medical Genetics and Genomics criteria, it is a well-established causal variant when in trans with specific alleles such as the p.Ala284Val and therefore is considered as a positive finding, which provides molecular diagnosis for the observed phenotype in the patient.
All ancestries with a total percentage amounting to less than 5% are combined into "Other."

INF2, NPHS2 COL4A4, and *COL4A5* (cases 4, 5, 9, and 10; Table 2). Variants in 1 gene (*INF2*) were diagnostic for autosomal dominant forms of FSGS, 1 (*COL4A4*) for autosomal dominant thin basement membrane disease and 1 (*COL4A5)* for X-linked Alport syndrome. One case carried a compound heterozygous pathogenic genotype at *NPHS2*. One patient carried a "G2" homozygous *APOL1* high-risk genotype associated to various forms of nondiabetic nephropathy, including FSGS in individuals of African ancestry,[26] as well as a novel *PAX2* variant of unknown significance, which is predicted to be deleterious.

Return of Results and Clinical Decision Making

The GS time from DNA isolation to completion of sequencing averaged 12.4 days (range 7–16 days), whereas the total turn-around time from enrollment to return-of-results and clinical decision averaged 21.8 days (range 15–42 days).

The genetic findings resulted in a change in diagnosis in 6 patients. In 5 of 6 patients (cases 4, 5, 6, 9, and 10) the etiological diagnosis was based on the genetic findings; in case 2, the negative genetic findings and clinical work-up prompted a re-evaluation of the kidney biopsy by the central pathologist, leading to a change in diagnosis from FSGS to scarred postinfectious glomerulonephritis (Table 3; Figures 1b and c). This change in diagnosis resulted in avoidance of unnecessary immunosuppression and formulation of a more favorable prognosis. Accordingly, the patient's kidney function remained stable at 2-years follow-up on angiotensin-converting enzyme inhibition alone. The discovery of a *COL4A5* variant in case 10 also prompted a re-evaluation of the kidney biopsy, and detailed analysis of electron microscopy showed thinning of glomerular membranes (Figure 1d), which was missed in the original pathology report. This resulted in a correction of diagnosis to X-linked Alport syndrome and obviated the need for a second biopsy, which had been planned in the event of a negative genetic test result, for evaluation of activity and chronicity as a guide to therapy. Conversely, case 4 had a kidney biopsy consistent with FSGS but with a glomerular basement membrane at lower limit of the normal thickness (Figure 1e). These findings, together with the negative family history and early age of onset, might have suggested a recessive form of Alport syndrome. GS, instead, identified a *de novo* heterozygous *INF2* variant, supporting the diagnosis of FSGS and refuting an autosomal recessive inheritance, thereby changing genetic counseling and optimizing transplant donor selection, as well as pretransplant and posttransplant immunosuppression. In fact, based on the genetics results, plasmapheresis pretransplantation and posttransplantation and B-cell depletion agents were not indicated, thus sparing significant additional and potentially harmful immunosuppression. As expected, after receiving a living-related kidney transplant, the patient did not undergo recurrence of FSGS. Furthermore, given the known association of *INF2*-related FSGS with Charcot-Marie-Tooth neuropathy and the localization of the p.Leu77Pro variant in the protein domain associated with Charcot-Marie-Tooth,[28] this patient underwent early screening (the same day of the return of results) for subclinical neurological disease and was found to have absence of most reflexes and mild distal weakness of the legs, suggesting early signs of Charcot-Marie-Tooth neuropathy despite an initially normal electromyography. On follow-up the patient then developed clinically and electromyographic overt peripheral neuropathy for which he receives regular

Table 3. Patient outcomes after return of results

Pt.	GS Time (d)	Total TAT (d)	Gene	Diagnosis Change	Clinical Decision	Suggested New Treatment	1-Year Follow-Up
1	13	17	Neg	No	New IS therapy	Rituximab	UPCR decreased to 2 mg/mg, sCreat 0.85 mg/dl, sAlb increased to 3.2 mg/dl; no edema.
2	8	15	Neg	Yes (new pathology dx)	- IS therapy not started; - Maintained on ACEi	No	Complete remission (UPCR 0.29 mg/mg, sCreat 0.7 mg/dl)
3	16	27	Neg	No	New IS therapy	CTX	-Rapid progression to kidney failure prior initiation of CTX; -Pre-emptive kidney tx - Post-tx plasmapheresis, Rituximab and Ofatumumab
4	13	15	*INF2*	Yes	- Steroid therapy not started; - Cautious initiation of tacrolimus because is suspected CMT; - Neurological work-up for CMT; - Maintained on ACEi	Tacrolimus	-On follow-up for CMT; -Tx evaluation for worsening renal function; -Started hemodialysis
5	13	20	*NPHS2*	Yes	- IS therapy not started; - Referred to sparsentan trial	Sparsentan	- Persistent NS; - Enrolled in clinical trial with sparsentan
6	13	15	*APOL1 (PAX2)*	Yes	- Steroids withdrawal; - IVIG therapy started for positivity to Parvovirus B19	IVIG	- Progression of renal disease; - Pre-emptive kidney tx
7	15	22	Neg	No	Maintained on current IS therapy (tacrolimus) with tapering plan	No	Stable complete remission of NS (sCreat 0.32 mg/dl, UPCR 0.14 mg/mg) on low dose tacrolimus
8	7	22	Neg	No	Maintained on current IS therapy (tacrolimus) with tapering plan of steroids	No	-Relapse after tapering off IS; -Remission on low dose tacrolimus after short course of steroids
9	11	23	*COL4A4*	Yes	Attempt with short-course steroids given the disproportionally high proteinuria for a single *COL4A4* variant	Steroids fast tapering	-Steroid-resistant persistent NS (UPCR 4.9 mg/mg) -Worsening renal function (sCreat 2.6 mg/dl)
10	15	42	*COL4A5*	Yes (new pathology dx)	No IS therapy or repeated biopsy	No	-Stable subnephrotic proteinuria

ACEi, angiotensin-converting enzyme inhibitor; *bx*, biopsy; *CMT*, Charcot-Marie-Tooth neuropathy; *CTX*, cyclophosphamide; *dx*, diagnosis; *GS*, genome sequencing; *IS*, immunosuppressive therapy; *IVIG*, intravenous immunoglobulin; *NS*, nephrotic syndrome; *Pt.*, patient; *sAlb*, serum albumin; *sCreat*, serum creatinine; *TAT*, turn-around time (from enrollment to return of results); *Tx*, transplant; *UPCR*, urine protein-to-creatinine ratio

physical therapy, and he is ambulating with braces and walker or in a wheelchair. In case 5, a 22-year-old male with positive family history of kidney failure, we identified a compound heterozygous genotype in *NPHS2*, composed of a known pathogenic variant (p.Ala284Val) and the high-frequency (∼3%) variant p.Arg229Gln. The p.Arg229Gln variant is atypical because it is relatively common in the population and becomes pathogenic depending on the trans-associated allele, as with the p.Ala284Val, which ultimately results in an altered heterodimerization and mislocalization of the encoded protein Podocin.[29] Pathogenic compound heterozygous genotypes with p.Arg229Gln usually result in delayed age of onset compared to classic recessive forms of NS, thus confounding the assessment of the possible mode of inheritance, and consequent genetic counseling, as in this case. Based on the genetic finding we recommended steroid avoidance and enrolled him in a clinical trial of sparsentan, a dual blocker of the endothelin receptor type A and the angiotensin receptor. Case 6, a 26-year-old self-declared White Hispanic male with NS caused by a collapsing form of FSGS and negative serology for HIV, was enrolled after being started on high dose steroids the week prior. GS revealed a homozygous *APOL1* G2 high-risk genotype and a novel deleterious variant in *PAX2*. *PAX2* pathogenic variants, traditionally considered as causal for congenital anomalies of the kidney and urinary tract, have been recently and increasingly identified in FSGS patients, even in absence of structural kidney anomalies or eye defects.[30,31] Although plausibility, absence in population controls, and constraints, would suggest this variant as a likely positive finding, we conservatively considered it as a variant of unknown significance. Altogether, these genetic findings prompted discontinuation of corticosteroid therapy. Moreover, given the known association of *APOL1* high-risk genotypes with viral infections and collapsing forms of FSGS,[32–37] a test for parvovirus was sent and resulted positive, leading to the initiation of treatment with intravenous immunoglobulin. This finding also confirms that, though highly correlated,[38] genetically determined ancestry is more accurate than self-declared race in guiding precision diagnosis of kidney diseases in which genetic risk estimates are strongly correlated with ancestral makeup.

Importantly, timely genetic evaluation allowed pretransplant and posttransplant immunosuppression optimization for case 3. This young patient with

progressive kidney disease, given the negative genetic testing, was considered at high-risk for disease recurrence post transplant and was treated pre-emptively with plasmapheresis and rituximab. Indeed, the patient developed early posttransplant recurrence of FSGS that was refractory to plasmapheresis and was then started on low-density lipoprotein apheresis reaching partial remission, followed by 6 weekly doses of ofatumumab. Proteinuria further decreased and stabilized (urinary protein-to-creatinine ratio 0.6 mg/mg) without the need of further plasmapheresis and her renal function remained normal for at least 3 years after transplantation.

In summary, both positive and negative GS findings determined a change of pharmacological treatment, including holding or stopping immunosuppression in 6 patients (cases 2, 4, 5, 6, 9, and 10), starting a new treatment in 5 patients (cases 1, 3, 4, 5, and 6) and maintaining the same immunosuppressive treatment in 2 patients (cases 7 and 8). In the 3 patients who approached or reached kidney failure (cases 3, 4, and 6), GS results were instrumental to the transplant evaluation by informing the screening of potential living-related donors and counseling for posttransplant management and disease recurrence. All patients and families received counseling for family planning based on genetic results.

Considerations About Study Design and Implementation

Here we provide a detailed roadmap for implementation of broad clinical genetic testing into kidney medicine practice that is designed to be completely embedded into standard-of-care outpatient nephrology visits.

As for any clinical test that is introduced as potential tool in the day-to-day clinical practice, there are several considerations to be made about the type of testing, the study design and sample size, and the applicability to different academic and nonacademic health systems. First, one might argue that exome sequencing or targeted panels might be more cost-effective than whole GS. Here we opted to test the implementation of GS rather than more focused capture approaches for several reasons; first, from a technical standpoint, without capture of exonic regions, the sample preparation for sequencing is faster, thus making it a test of choice when results are needed in a timely fashion; second, GS results in uniform sequencing of both coding and noncoding regions, thus providing high sensitivity for variant detection; third, GS allows for assessment of copy number variations and other structural variants that are often difficult to capture with targeted panels; fourth, the costs of GS are continuing to decrease, thus making it the most comprehensive and cost-effective genetic test[39–42] that can be conducted once in a lifetime and reanalyzed and reinterrogated at timed-intervals during follow-up while new genomic discoveries are made. Finally, recent advances in human genetics studies that are aimed at assessing the contribution of genome-wide polygenic risk scores to common and rare conditions suggest that clinically relevant polygenic risk scores will be increasingly introduced in clinical practice to ascertain risk, prognosis, and guide medical management.[43–45] Estimates of individual genome-wide polygenic risk scores require accurate genotyping of millions of variants to which targeted or exome panels are blind to. On the contrary, GS allows, in a single test, to directly capture all variants required to compute such risk scores. In conclusion, when deciding about implementing a novel test in the real-time outpatient medical management of kidney disease, GS represents the most comprehensive, flexible, durable, and cost-effective tool, especially considering the rapid advancement not only in gene discovery but also on the interpretation of the contribution of common noncoding variants and the polygenic background to rare and common diseases.

Sample size may be perceived as a limitation; however, this is typical of pilot studies. Moreover, specific to this design, considerations regarding implementation and feasibility would not differ for either smaller or larger studies. The limited number of patients recruited, and the narrow inclusion criteria have increased on purpose the rate of diagnostic findings as a proof of concept to assess feasibility of return of results, therefore overestimating the clinical impact of rapid GS for proteinuric kidney diseases at large. Nevertheless, the goal of this study was not to show utility but to demonstrate that introducing rapid clinical GS in outpatient nephrology care is achievable, regardless of the diagnostic yield. Although we demonstrated feasibility, with margins for improvement, the implementation of such approach into clinical practice at centers where limited resources exist might be challenging. Indeed, conducting counseling pre- and post- genetic testing, GS analysis and interpretation, and multidisciplinary return of results to allow a time-sensitive clinical decision is likely to be difficult in nonacademic centers and or in the community at the current time. We do not perceive this issue as different from any other advanced diagnostic or therapeutic approach that is first introduced in medical centers of excellence and, when optimized and streamlined, deployed at large in the community.

DISCUSSION

Clinical management of proteinuric kidney diseases and, in particular, FSGS, has proven to be challenging, often involving a repeated trial-and-error strategy to identify the appropriate pharmacotherapy and heavily relying on individual clinical experience. As a result, many patients undergo prolonged, and often unnecessary, immunosuppression courses, and only in a few instances do they receive specific etiological treatment for their kidney disease or the possible associated extrarenal manifestations. The lack of a genetic diagnosis at the time of clinical presentation or during the course of medical management when knowledge of the genetic cause (or absence of it) matters, hampers such precision medicine approaches, makes genetic counseling and family planning imprecise, and impedes a complete transplant evaluation and donor selection. Here we report on a pilot study designed to implement and evaluate the feasibility and impact of rapid, Clinical Laboratory Improvement Amendments–certified, GS on the clinical management of children and young adults with biopsy-proven proteinuric kidney disease in the real-world outpatient nephrology settings. We showed that a multidisciplinary approach involving clinicians, geneticists, and pathologists combined with rapidly delivered and clinically-certified genetic results, can be successfully implemented and can significantly impact diagnosis and clinical decision, including treatment, counseling, and transplant evaluation optimization.

Previous studies have shown the feasibility and impact that rapid genetic diagnosis can have on neonatal or pediatric conditions.[46] Presently, many healthcare systems routinely implement GS in the neonatal and pediatric inpatient setting, whereas clinical GS is not yet used for outpatients or adults. In fact, these studies were conducted on highly-selected populations not only with a high pretest probability for genetic disorders, but also in a very controlled setting, mostly in neonatal or pediatric intensive care units, where logistics about consenting, enrollment, samples collection, return of results, and consequent counseling and therapeutic adjustments can be achieved in a relatively easier fashion than what needs to be accomplished in an outpatient setting.[21,46] The workflow in our study required close interaction among researchers, geneticists, clinical coordinators, physicians, and pathologists, with additional effort to fully embed genetics into routine nephrology care. Nevertheless, the total turn-around time from enrollment to return of results and clinical action averaged 21.8 days, which is only slightly longer than those reported in neonatal and pediatric intensive care units, and it is well within a critical window by which genetic-driven strategy is clinically meaningful for the individual patient with proteinuric kidney disease.

In conclusion, here we show that rapid GS at the time of kidney biopsy diagnosis or during the course of standard-of-care nephrology visits in which treatment planning requires knowledge of a patient's genetic status, is feasible and can be implemented in the day-to-day outpatient care. This framework has the potential of helping guidance in clinical management and improving patient outcome as follows: by (i) achieving and optimizing diagnosis; (ii) sparing ineffective and toxic immunosuppressive treatment in unresponsive genetic forms of disease; (iii) identifying genetic causes that might be amenable to etiologic therapy; (iv) supporting the indication for appropriate second and third line immunosuppressive treatment in nongenetic forms of disease; (v) guiding screening for renal and extrarenal manifestations; (vi) informing transplant evaluation and management, including related living donor options, thus optimizing organ allocation and outcome, as well as plasma exchange treatment decision; and (vii) enabling early genetic counseling, cascade testing, and family planning. We show that all of these outcomes can be accomplished in a real-world outpatient setting within 3 weeks of a diagnostic biopsy or a critical clinical question.

Additional multicenter studies are needed to confirm the utility of rapid clinical GS in clinical management across age, race or ethnicity, and a broader phenotypic spectrum of NS/FSGS patients. Such studies will enable the stratification of patients for clinical trials and the assessment of cost-effective analysis to prove diagnostic utility of rapid GS, which should facilitate reimbursement of genetic tests by third party payers.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENT

Funding

This research is supported by the Department of Defense (PR190746, PR212415), by the National Center for Advancing Translational Sciences, National Institutes of Health (Grant Number UL1TR001873) and by the National Institute of Health Grant RC2DK122397. A.M. received support by the American Society of Nephrology KidneyCure Ben J. Lipps Research Fellowship. JGN received support by the National Institute of Health fellowship TL1TR001875.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Detailed description of all ten cases and their clinical course.

Supplementary Reference.

Figure S1. Study design and outcomes.

Table S1. List of genes ($N = 126$) associated to Mendelian forms of nephrotic syndrome (NS)/focal segmental glomerulosclerosis (FSGS) or phenocopies of NS/FSGS.

Table S2. List of genes ($N = 552$) associated to Mendelian forms of non- NS/FSGS kidney disease.

STARD Checklist.

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