HHS Public Access

Author manuscript

Am J Kidney Dis. Author manuscript; available in PMC 2024 June 06.

Published in final edited form as:

Am J Kidney Dis. 2016 June ; 67(6): 992–993. doi:10.1053/j.ajkd.2015.11.023.

A Custom Targeted Next-Generation Sequencing Gene Panel for the Diagnosis of Genetic Nephropathies

Christopher P. Larsen, MD[1], **Tim Durfee, PhD**[2], **Jon D. Wilson, MD**[1], **Marjorie L. Beggs, MD**[1]

[1]Nephropath, Little Rock, Arkansas

[2]DNASTAR, Madison, Wisconsin

To the Editor:

Traditional strategies for determining the precise genetic cause of kidney disease are costly and time consuming. However, recent advances in high-throughput sequencing have dramatically decreased the cost, time, and labor involved. With new data emerging about the role of genetics in kidney disease, realizing the era of personalized and precision medicine will require a precise diagnosis and identification of the underlying molecular pathogenesis.[1,2] We describe a project to design and validate a next-generation sequencing (NGS) panel for the full spectrum of genetic nephropathies in a CLIA-approved laboratory and to test it in a series of kidney biopsies with unknown molecular pathogenesis.

A custom targeted NGS gene panel was designed covering 301 genes described in kidney disease (the full list, which may be helpful for others developing similar panels, is provided in table a of Item S1). Validation runs showed accurate results, with a mean of >99% coverage of the targeted regions at a depth greater than 20×. Performance characteristics of the assay were excellent (table b of Item S1), based on comparison to a reference genome and Sanger sequencing.[3]

After assay validation, we sequenced extracted DNA from kidney biopsy samples under an IRB-approved protocol (Schulman Associates IRB). We sought to interrogate cases in which the pathogenesis remained unknown after kidney biopsy. Fifty consecutive kidney biopsies with adequate DNA that met inclusion criteria were sequenced. This included patients younger than 40 years, with biopsy evidence of either idiopathic FSGS or cases without FSGS that had at least moderate chronic injury with no discernable cause either by biopsy or

Corresponding author: chris.larsen@nephropath.com.

Contributions: Research idea and study design: CPL, JDW, MLB; data acquisition: MLB; data analysis/interpretation: CPL, JDW, TD, MLB; statistical analysis: TD, MB; supervision or mentorship: CPL, TD. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. CPL takes responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Financial Disclosure: The authors declare that they have no relevant financial interests.

Supplementary Material

Item S1. Detailed methods, genes included in the panel, variant call precision and reproducibility, cases with an identified monogenic etiology by race, detailed clinical/genetic findings of the 50 kidney biopsies.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2015.11.023) is available at www.ajkd.org

clinical parameters. Variants identified were classified according to relevant guidelines.[4] We were able to identify pathogenic or likely pathogenic variants in 23 patients (Table 1). Five patients had a known family history of kidney disease, including 3 with FSGS on biopsy and 2 with CKD, NOS. Of those with a family history, 4 of 5 had an identified genetic cause for their kidney disease. All pathogenic variants were validated by a second modality such as *APOL1* genotyping or Sanger sequencing, with 100% accuracy.

In both the FSGS and idiopathic CKD groups, >40% of the participants studied had evidence of a monogenic cause for their kidney disease. There was an especially large genetic disease burden among the African Americans studied, secondary to *APOL1* risk alleles (table c of Item S1). We found that this is by far the largest contributor to genetic kidney disease in our patients and is responsible for nephropathy more than twice as often as all other genes combined (Table 1). Outside of African Americans, we identified only one patient (identified as Hispanic) who harbored one *APOL1* risk allele. Information for patients' clinical details and genetic diagnosis is presented in table d of Item S1.

The pattern of FSGS on biopsy in the primary FSGS group was not predictive of the presence of genetic abnormalities. Of 22 (61%) cases that showed a nonspecific NOS FSGS pattern, 9 (41%) had pathogenic variants identified by the panel, including 5 (23%) with *APOL1* risk alleles. The rest included 2 cases with pathogenic variants in *INF2* and 1 case each of *COL4A4* and *COL4A5* variants. Twelve cases showed a collapsing pattern of FSGS (33%). Seven (58%) of these cases had evidence of a genetic cause, including 6 (50%) due to *APOL1* risk variants. Overall, cases with the collapsing pattern of FSGS were more likely to have a genetic cause than those with an NOS pattern, but the difference was not significant ($P = 0.5$).

Genetic testing enabled a specific molecular pathogenesis-based diagnosis in 46% of biopsies studied. This more specific genetic diagnosis provides important information for a number of reasons. Some data suggest that these patients might be less likely to respond to immunosuppressant medications.[5] If verified, patients with an identified genetic cause could potentially be spared long-term intensive immunosuppressant regimens with the accompanying serious adverse side effects. Moreover, if specific treatment were to become available, the patient would not know they were eligible without knowledge of what is causing the disease. There was 1 patient with *COQ2*-associated FSGS in which the genetic diagnosis indicates the need for a specific treatment.[6] Also, knowledge of the specific cause often offers peace of mind for patients, and a genetic diagnosis provides opportunities for genetic counseling and family planning decisions.

Testing this cohort provided us with important information about our kidney biopsy population and also enabled us to validate the NGS assay on DNA extracted from kidney biopsy tissue. Our results suggest that a panel-based approach using NGS is an efficient and cost-effective approach to the diagnosis of genetic nephropathies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Support: This research was supported by NIH/NIDDK grant R43DK103432.

References

1. McCarthy HJ, Bierzynska A, Wherlock M, et al. Simultaneous sequencing of 24 genes associated with steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol. 2013;8:637–648. [PubMed: 23349334]
2. Sadowski CE, Lovric S, Ashraf S, et al. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. J Am Soc Nephrol. 2015;26:1279–1289. [PubMed: 25349199]
3. Zook JM, Chapman B, Wang J, et al. Integrating human sequence data sets provides a resource of benchmark SNP and indel genotype calls. Nat Biotechnol. 2014;32:246–251. [PubMed: 24531798]
4. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–424. [PubMed: 25741868]
5. Giglio S, Provenzano A, Mazzinghi B, et al. Heterogeneous genetic alterations in sporadic nephrotic syndrome associate with resistance to immunosuppression. J Am Soc Nephrol. 2015;26:230–236. [PubMed: 25060053]
6. Diomedi-Camassei F, Di Giandomenico S, Santorelli FM, et al. COQ2 nephropathy: a newly described inherited mitochon-driopathy with primary renal involvement. J Am Soc Nephrol. 2007;18:2773–2780. [PubMed: 17855635]

Table 1.

Clinicopathologic Summary and Likely Genetic Cause of Kidney Disease, by Clinical Presentation

	Total (N = 50)	CKD, NOS (n = 14)	FSGS (n = 36)
Age, y	24.2 [2-38]	26.3 [12-38]	23.4 [2-37]
Male sex	24	5	19
Race			
African American	21 (42%)	6 (43%)	15 (42%)
White	15 (30%)	3 (21%)	12 (33%)
Hispanic	11 (22%)	4 (29%)	7 (19%)
Other	3 (6%)	1 (7%)	2 (6%)
Serum creatinine, mg/dL	3.8	3.6	3.9
Proteinuria, g/d	5.4	2.2	6.6
Pathogenic/likely pathogenic variant identified	23/50 (46%)	6/14 (43%)	17/36 (47%)
Gene with pathogenic/likely pathogenic variant			
APOL1	16/50 (32%)	5/14 (36%)	11/36 (31%)
INF2	2/50 (4%)	0	2/36 (6%)
COL4A5	1/50 (2%)	0	1/36 (3%)
COL4A4	1/50 (2%)	0	1/36 (3%)
NPHP1	1/50 (2%)	1/14 (7%)	0
COQ2	1/50 (2%)	0	1/36 (3%)
CFI	1/50 (2%)	0	1/36 (3%)

Note: Values shown are mean [range], number (percentage), or n/N (percentage).

Abbreviations: CKD, chronic kidney disease; FSGS, focal segmental glomerulosclerosis; NOS, not otherwise specified.