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Genetic testing for nephrotic syndrome and FSGS in the era of next-generation sequencing

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Abstract

The haploid human genome is composed of three billion base pairs, about one percent of which consists of exonic regions, the coding sequence for functional proteins, also now known as the “exome”. The development of next-generation sequencing makes it possible from a technical and economic standpoint to sequence an individual’s exome but at the cost of generating long lists of gene variants that are not straightforward to interpret. Various public consortiums such as the 1000 Genomes Project and the NHLBI Exome Sequencing Project have sequenced the exomes and a subset of entire genomes of over 2500 control individuals with ongoing efforts to further catalogue genetic variation in humans.¹ The use of these public databases facilitates the interpretation of these variant lists produced by exome sequencing and, as a result, novel genetic variants linked to disease are being discovered and reported at a record rate. However, the interpretation of these results and their bearing on diagnosis, prognosis, and treatment is becoming ever more complicated. Here, we discuss the application of genetic testing to individuals with focal and segmental glomerulosclerosis (FSGS), taking a historical perspective on gene identification and its clinical implications along with the growing potential of next-generation sequencing.

What is FSGS?

FSGS is not a disease. Rather, it is form of kidney injury defined by histology. Studies of rare, monogenic forms of FSGS and nephrotic syndrome have yielded significant insight into our knowledge of the glomerular filter. These investigations have informed our current understanding of FSGS as a podocyte disorder and in some cases helped identify entirely new molecules with critical roles in glomerular function. The tendency for clinicians to use different categories of classification of clinical syndromes such as steroid resistant nephrosis or histopathologic entities such FSGS, to label patients as if they are both actual disease entities does a disservice to the accurate description of disease.

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DISCLOSURE

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Genetic studies in monogenic forms of disease

Positional cloning studies in neonates and their families with congenital nephrotic syndrome of the Finnish type identified mutations in a previously unknown gene, *NPHS1* or nephrin, as the underlying cause.² The encoded transmembrane protein serves both structural and signaling roles.³ Inheritance of two pathogenic nephrin mutations was initially described as a cause of massive proteinuria at birth, loss of podocyte foot process architecture, obliteration of the slit diaphragm, and characteristic microcystic dilatation of the proximal tubules.²

Subsequent genetic studies identified mutations in another slit diaphragm protein, *NPHS2* (podocin), as a relatively common cause of autosomal recessive steroid resistant nephrotic syndrome (SRNS) in early childhood.⁴ In contrast to patients with nephrin mutations, individuals with two pathogenic *NPHS2* mutations generally present with nephrotic syndrome between 3 months and 6 years of life, with some individuals presenting with congenital nephrosis and others with adult onset FSGS.^{5, 6} Certain podocin mutations appear to be more likely to cause symptoms at an earlier age, particularly frameshift, nonsense, or homozygous p.R138Q missense mutations.^{7–12} *NPHS2*-mediated disease is resistant to corticosteroids and other immunosuppressants and generally progresses to ESKD, and have a reduced risk for recurrence of FSGS after kidney transplantation.^{4, 9, 13, 14} Podocin is now understood to be a lipid raft component of the slit diaphragm that is essential for nephrin localization.^{15, 16}

Genotype-phenotype correlation studies suggest that progression to ESKD is more rapid in patients with pathogenic *NPHS1* mutations compared to those with *NPHS2* mutations.^{5, 17, 18} *NPHS1* manifests congenitally while *NPHS2* disease onset generally is in early childhood with development to end-stage kidney disease before 10 years of age.^{7–9, 19} Homozygous or compound heterozygous mutations in *NPHS2* have been found to cause disease in 6–17% of sporadic cases and 28–39% of familial cases of SRNS. A notable caveat occurs in individuals who carry the common p.R229Q *NPHS2* polymorphism in conjunction with a disease-causing mutation.^{6, 11} In this circumstance, the presence of a single disease-causing mutation along with this common polymorphism appears sufficient for kidney disease to manifest, but it is less severe with later ages of onset compared to those who carry two disease-causing alleles. There are also a significant fraction of cases of congenital SRNS due to pathogenic *NPHS2* mutations and therefore both *NPHS1* and *NPHS2* should be examined when a genetic diagnosis is attempted in congenital disease.^{5, 12, 18, 20, 21}

Though mutations in *NPHS1* and *NPHS2* represent the commonest genetic causes of non-syndromic SRNS congenitally, in infancy and childhood, other gene culprits have been identified including Wilms' tumor 1 (*WT1*), *LAMB2* (laminin beta 2), and *PLCE1* (phospholipase C epsilon). Of these, *WT1*, a key kidney development gene, is the best studied. Mutations in *WT1* lead to syndromic forms of kidney disease: Frasier syndrome and Denys Drash Syndrome. *WT1* mutations can also rarely cause isolated nephrotic syndrome in an autosomal dominant manner.^{22–26} Frasier syndrome is characterized by SRNS in childhood with histologic findings of FSGS, and slow progression to ESKD within the

second and third decades in life, male pseudohermaphroditism and a high incidence of gonadoblastomas. Denys Drash syndrome is characterized by SRNS earlier in life, during infancy with histologic findings of mesangial sclerosis and rapid progression to ESKD, ambiguous genitalia and nephroblastoma (Wilms' tumor). A study of 52 patients from 51 families with nephrotic syndrome due to *WT1* led to a number of genotype-phenotype correlations.²⁷ With respect to nonsyndromic forms of disease, mutations involving the three amino acids KTS (lysine, threonine, serine) in the splice site in intron 9 can cause isolated nephrotic syndrome with the absence of Wilms' tumor in 46, XX phenotypically concordant females.²⁴ Mutations missense in exons 8 and 9 have been also detected in patients with isolated diffuse mesangial sclerosis (DMS).²⁸ Pathogenic mutations in *PLCE1* lead to congenital or early onset nephrotic syndrome in an autosomal recessive fashion.^{29–31} There are a few reported individuals with *PLCE1* mutations responding to immunosuppressive therapy. Homozygous mutations in *LAMB2* can lead to isolated nephrotic syndrome or as part of Pierson syndrome, characterized additionally by ocular and neurologic abnormalities.^{32–34} Unlike the other genes described, *LAMB2* encoded protein is deposited by the podocyte into the GBM. Pathogenic mutations in *WT1*, *PLCE1* and *LAMB2* can manifest histologically as FSGS or diffuse mesangial sclerosis.

More recent studies utilizing next-generation sequencing technology and involving consanguineous families have identified other autosomal recessive childhood SRNS genes including *MYO1E* (encoding myosin 1E), *NEIL1* (encoding Nei endonuclease VIII-like 1), *CUBN* (encoding cubilin), and *ARHGDI1* (encoding RhoGDI alpha).^{35–39} The frequencies of mutations in these genes in congenital and steroid nephrotic syndrome is unclear.

In 2000, the genetic study of three kindreds with adult onset autosomal dominant FSGS identified mutations in alpha-actinin 4 (*ACTN4*), a cytoskeleton actin binding protein, as the cause of disease.⁴⁰ In 2005, mutations in *TRPC6*, transient receptor potential cation channel, subfamily C member 6, were shown to cause a similar autosomal dominant form of FSGS.⁴¹ Though both *ACTN4* and *TRPC6* are ubiquitous proteins found throughout the body, pathogenic mutations in these genes appear to only affect kidney function. More recently, mutations in inverted formin 2 (*INF2*), another cytoskeleton actin binding protein, were identified as another cause of autosomal dominant FSGS.⁴² The discoveries of *ACTN4* and *INF2* underscored the importance of the actin cytoskeleton in the maintenance of the molecular machinery in the podocyte.⁴² In contrast to *ACTN4* and *TRPC6*, *INF2* mutations can cause not only isolated FSGS, but FSGS together with the neuromuscular disorder, Charcot Marie Tooth disease.⁴³

With respect to adult onset familial FSGS, pathogenic mutations in *INF2*, *TRPC6* and *ACTN4* account for disease in 9%, 3% and 2% of the cohort of approximately 200 autosomal dominant families that we have studied^{44–46}. These genes are rarely found to contribute to disease in our cohort of sporadic FSGS. Other groups have reported *INF2* to cause FSGS in 12–17% of families with monogenic disease.^{42, 47, 48} We have not observed mutations in other reported adult onset FSGS genes including *CD2AP* and *PLCE1* in our own family cohort (unpublished results).

The autosomal dominant forms of FSGS tend to have more varied penetrance and clinical presentation than autosomal recessive forms of nephrotic syndrome, presenting at later ages, with different severities of disease, suggesting different mechanisms of podocyte injury. Given the rarity of these monogenic diseases, there is little published data regarding precise genotype-phenotype correlations. This is not dissimilar to observations with other (renal and non-renal) autosomal dominant diseases, where onset often occurs in adulthood and severity of expression of disease is variable and likely depends on both other genetic as well as environmental factors. For example, our own observations suggest that families with *INF2* mutations manifest proteinuria, particularly microalbuminuria in childhood, although the affected individuals usually first come to medical attention in the mid-teens to middle age. ESRD is common but not absolute, developing anywhere from the mid-teen years to late middle age.^{42, 47, 48} It is unknown if medications such as angiotensin converting enzyme inhibitors can prevent or delay the onset of ESKD in these patients.

Risk alleles in African Americans with kidney disease

FSGS occurs with higher frequency in African Americans compared to Europeans and European Americans. As a result of this observation, two genome wide association studies (GWAS) using mapping by admixture methods identified a genetic susceptibility locus on chromosome 22 near MYH9.^{49, 50} Subsequently studies identified variation in the *APOL1* gene as the likely causal variants driving this association.^{51, 52} Specifically, two coding variants, named G1 and G2, in the *APOL1* gene were discovered that conferred a significant risk of developing FSGS, hypertension-associated kidney disease (H-ESKD), or HIV nephropathy (HIVAN).^{51, 52} Individuals who are either homozygous or compound heterozygous for the risk alleles have a staggering 7–10 fold increased risk of developing FSGS or H-ESKD. The risk of HIV associated kidney disease is even greater: individuals with two *APOL1* risk alleles have an odds ratio as high as 30 for developing FSGS.^{51, 53, 54} The two risk alleles are common in individuals of African ancestry, with a combined allele frequency of approximately 35% in African Americans.⁵⁴ Despite the negative impact on kidney function, the G1 and G2 variants may have been propagated over time due to enhanced lytic activity against the African Sleeping Sickness pathogen *Trypanosoma b. rhodesiense*, leading to positive natural selective pressures on alleles bearing these polymorphisms. The mechanism by which these variants in *APOL1* cause kidney disease remains unknown. Interestingly, a study involving 136 kidney transplant recipients, 22 of which received deceased donor kidneys with two high risk *APOL1* variants suggests that these grafts fail more rapidly.⁵⁵ The recipient *APOL1* genotype, by contrast, does not seem to affect the transplant outcome.⁵⁶ Though this study needs replication in a larger cohort, these results suggests a factor intrinsic to the kidney plays a role in disease pathogenesis. It also raises the question of whether *APOL1* testing should be part of the routine pre-transplant workup for potential African American kidney donors.

The genes discussed above represent a subset of the best understood genes reported to underlie the development of non-syndromic FSGS and nephrotic syndrome (Table). We refer the reader to other recent reviews with more in-depth discussion of some additional genetic causes.^{7, 57}

Treatment of monogenic nephrotic syndrome and FSGS

A major clinical challenge in the management of nephrotic syndrome and FSGS involves the decision to use corticosteroids and other immunosuppressive agents. Retrospective cohort studies and case reports suggest that patients with monogenic forms of FSGS and nephrotic syndrome are much less likely to respond to these agents. One of the largest studies to examine this issue was by Buscher et al who evaluated the response to cyclosporine treatment in 43 study patients.⁵⁸ Two patients with *WT1* mutations had a partial response to cyclosporine, but one nonetheless eventually progressed to ESKD. Occasional partial responses have been observed in various other forms of inherited FSGS/SRNS.^{59–61} None of the other genetic cases of congenital nephrotic syndrome or SRNS responded to cyclosporine. By contrast, 68% of the patients without a known genetic cause responded to this treatment.

A similar study was performed by Ruf et al and included 190 patients with SRNS and 124 individuals with steroid sensitive nephrotic syndrome (SSNS).¹³ Pathogenic *NPHS2* mutations were found in 26% of individuals with SRNS but not those with SSNS. FSGS pathology was found in 61% of patients with SRNS and in 21% with SSNS. 17% of individuals with *NPHS2* disease had a partial response to corticosteroids or cyclosporine but none had a complete response.

Angiotensin converting enzyme inhibitors or angiotensin receptor blockers have not been adequately studied in genetic forms of nephrotic syndrome. However, given their relatively benign side effect profile as compared to immunosuppressive agents, it seems reasonable to treat patients with genetic forms of FSGS or SRNS with these medications.

Thus, the published data regarding the use of various therapies in genetic kidney disease is sparse and, by virtue of incomplete information, inconclusive. We therefore provide a largely opinion-based recommendation. Although there are exceptional patients who do respond to immunosuppression, they are rare and the response is partial. The majority of patients with disease-causing *NPHS1* and *NPHS2* mutations do not respond to corticosteroids or other immunosuppressant agents. We believe that immunosuppressive medications are not indicated in this setting and genetic testing when these diagnoses are suspected as important. *NPHS1* and *NPHS2* mediated disease should be considered in congenital, infant and childhood onset nephrotic syndrome, regardless of family history. If immunosuppressive agents are to be used nevertheless, genetic testing is still important as it may guide the aggressiveness of therapy. The autosomal dominant forms of FSGS caused by pathogenic mutations in *TRPC6*, *ACTN4*, and *INF2* have not been well studied with regard to response to these medications. These individuals with overt disease should be placed on ACEi and/or ARB, unless a contraindication exists. We note that KDIGO guidelines suggest that some adults with genetic FSGS have responded to immunosuppressive treatment and thus should be tried.⁶² It is not known whether at-risk but pre-symptomatic patients would benefit from early treatment with any of these agents.

The future of discovery approaches

The classical approach to gene discovery in monogenic disease has been linkage analysis to localize a disease locus, followed by fine mapping and ultimately gene identification. The success of such investigation has been based on studies in genetically informative kindreds. In such linkage studies, genome-wide genotyping of informative markers leads to an unbiased assessment of the genome for disease associated haplotypes. In the past, it has been helpful to narrow the relevant genetic region to a manageable length for directed Sanger sequencing. In the case of congenital nephrotic syndrome of the Finnish type, for example, genetic studies narrowed this critical region to 150 kilobases, facilitating identification of the nephrin gene.² Biased approaches, such as sequencing selected candidate genes chosen on the basis of known biology, have had more limited success.

The recent advent of next-generation sequencing has unveiled enormous potential, allowing a single advanced sequencing machine to read billions of base pairs within a week. This is in stark contrast to Sanger sequencing where one reaction targets a specific amplified segment of DNA measuring several hundred base pairs long. Thus, these small to moderate sized families that were previously insufficiently informative genetically are now amenable to genetic analysis. As a result of clever application of this new technology, several new genes have been identified as causing recessive forms of syndromic and non-syndromic nephrotic syndrome.^{35, 37}

Data examining variants in single families with a presumed inherited disease need to be interpreted with caution. The natural variation in the human genome is significant and determining whether a specific genetic variant causes disease is challenging, especially given that most genes lack easy, high throughput functional assays to establish in vitro or in vivo evidence of variant pathogenicity. For example, in podocin alone, over 100 pathogenic mutations have been described, along with many other variants of unknown significance.⁷ Nonetheless, the falling prices of exome and next-generation sequencing will make it possible in the very near future to sequence all of an individual's coding sequence easily, raising a variety of new questions in both the research and clinical laboratories.

Genetic testing: When and how should we do it?

Below, we first focus on more traditional forms of genetic testing, as currently implemented by the majority of clinical laboratories. We then discuss newer methods of genetic testing, such as whole exome, whole genome sequencing, and targeted sequencing of gene panels.

Clinical utility of genetic testing

Like any other clinical tool, physicians and patients need to consider the pros and cons of any specific genetic test. There are several potential benefits for both the patient and his/her physician in performing a genetic test. Genetic testing in the context of FSGS/NS may be able to provide the following: 1. Aid in diagnosis, especially a “fuzzy” phenotype such as FSGS with clinical features common to several forms of kidney injury; 2. Limiting medication exposure, or identifying a “best” treatment; 3. Help in determining risk of recurrent disease in kidney transplantation; 4. Allowing for risk assessment in candidate

living related kidney donors; 5. Aid in prenatal diagnosis. 6. Motivate screening in other members of a family.

The clinical utility of genetic testing at present is particularly relevant to childhood onset SRNS. Childhood onset disease is often divided further by ages of onset into the following categories: 1. congenital nephrotic syndrome (age <3 months); 2. infant (3 months –12 months); 3. early childhood (13 months – 5 years); 4. late childhood (6–12 years); 5. adolescence (13–18 years). Studies have shown that disease-causing mutations can be identified in the vast majority of cases of congenital nephrotic syndrome, accounting for disease in 80 to 100% of cases, even in the absence of a family history.^{5, 17} However, the likelihood of identifying a monogenic etiology falls with older age of onset of disease. A recent study by Santin *et al* examined 125 patients belonging to 110 families with congenital, infant, child and adult onset nephrotic syndrome, SRNS, diffuse mesangial sclerosis and FSGS.¹⁷ A genetic cause was identified in 100% of patients with congenital nephrotic syndrome and in 57% of infantile onset disease. With older age of disease onset, defects in known disease genes cause fall further. The likelihood of finding a monogenic etiology was significantly higher in familial cases (67%) compared with sporadic cases (25%). Overall, disease-causing mutations were identified in 34% of cases with SRNS due to the following culprits: *NPHS1* 13.5%, *NPHS2* 12%, *WT1* 4.5%, *TRPC6* 3%, *INF2* 1%. A similar recent study by Buscher *et al* studied 91 patients from 82 families and found disease-causing mutations in 52%.⁵⁸ Twenty-six patients had congenital nephrotic syndrome and disease-causing mutations were identified in all of these patients. All but one patient with *NPHS1* associated disease presented congenitally. The majority of individuals with *NPHS2* mutations presented between 2.5 and 12 years of age, with the exception of three patients who had congenital nephrotic syndrome. Mutations in *WT1* and *TRPC6* were also reported in childhood onset disease. Congenital nephrotic syndrome can also be identified as part of several syndromes resulting from mutations in *LAMB2*, *PSSD2*, and *COQ2*.¹⁸ Given that congenital nephrotic syndrome is most commonly due to *NPHS1*, *NPHS2* and *WT1*, these are the best studied.

Indications for genetic testing

When should genetic testing be considered and what genes should be screened? Whole exome sequencing is increasingly being applied as a clinical tool. Obtaining unfiltered lists of variants in hundreds or thousands of genes, however, is not useful clinically. Many genes will have coding sequence variants, some of which will be rare or novel when compared to control sequence data. It is a non-trivial challenge to determine which, if any of these, are pathogenic. For research purposes, variant pathogenicity can be predicted by models that consider a protein's structure and by *in vitro/in vivo* functional assays. Prediction models, however, are far from perfect. Functional assays of gene and the gene product may be difficult, time consuming, and of uncertain relevance to the phenotype of interest.⁶³ Our knowledge is most complete for genes responsible for congenital nephrosis, including *NPHS1*, *NPHS2* and *WT1*, where these genes account for essentially all disease. In childhood onset nephrotic syndrome, known genes account for a substantial percentage, though clearly not all, disease. Therefore, at the present time, genetic testing for clinical purposes is most useful in congenital and childhood onset SRNS, regardless of family

history. The genes *NPHS1*, *NPHS2*, *WT1*, *LAMB2* and *PLCE1* should be included in any comprehensive testing of childhood nephrotic syndrome. In adult-onset FSGS where the individual has a family history of disease, genetic testing, if felt clinically indicated, should include the commonest genetic causes such as *INF2*, *ACTN4* and *TRPC6*. *NPHS2* disease can also manifest in either adolescence or adulthood if the common p.R229Q is present and so we include this as part of the diagnostic tests that should be considered in adults as well. An additional difficulty comes from the observation that any form of familial (or non-familial) kidney disease that leads to secondary glomerulosclerosis can be mislabeled as idiopathic FSGS. To be truly exhaustive, testing would need to include all genes leading to such phenotypes, such as the many nephronophthisis genes.⁶⁴ Though clinically approved diagnostic tests currently rely on sequencing by the Sanger method, genetically heterogeneous conditions like SRNS and monogenic FSGS are particularly amenable to sequencing by next-generation methods whereby a large number of genes can be examined. A recent study sequencing 24 known genes associated with SRNS in 36 pediatric patients by next-generation method suggests that this technology is accurate and sensitive.³¹ When clinical use of next-generation sequencing becomes commonplace, cases where no known gene culprit is identified will pose challenges and dilemmas in clinical interpretation of rare variants but nevertheless will be useful for research purposes.

Genetic testing in transplant

Genetic testing may also be helpful in assessing the risk of FSGS recurrence in a transplanted kidney. Individuals with pathogenic *NPHS2* mutations are significantly less likely to have recurrence of their disease in a transplanted kidney when compared to individuals with primary FSGS.^{4, 13, 14} Patients with an established genetic diagnosis may be able to avoid some of the pre- and post-transplant therapies designed to prevent FSGS recurrence. To date, we do not know of any transplant recurrence in individuals in our database with *ACTN4*, *TRPC6*, or *INF2* mutations (our unpublished data). Genetic testing can also aid in choosing a living kidney donor. In cases where the kidney recipient has an autosomal dominant genetic mutation that appears likely to be causative for his or her disease, living donors can be screened to ensure that they are not silent carriers of that mutation. In this way, one can avoid putting both the recipient and the donor at risk for disease.

Benefits and risks of genetic testing to the individual and family

Genetic testing can also be helpful to the clinically unaffected members of a family with hereditary nephrotic syndrome. Once a genetic diagnosis has been made for one affected individual, the other relevant members of the family can be tested efficiently and accurately. Those who also carry the mutation(s), especially in autosomal dominant disease, can be followed more closely for development of proteinuria or hypertension, and may benefit from earlier intervention with ACEi/ARB, although this notion is not supported by any published data. Individuals in families with hereditary FSGS, particularly autosomal dominant forms, may be faced with the anxiety of wondering if they or their children will develop FSGS. Genetic testing can provide reassurance to those who are shown to be negative for the mutation(s) that run in their family but can also provide useful information regarding risk

and family planning measures for those who are carriers. Prenatal testing is another option to discuss as well.

There are potential emotional and psychological risks to the patients and their family to consider with genetic testing. A positive test can cause feelings of distress and guilt in a parent who harbors the disease-causing mutation and has passed it on to his or her children, especially in cases where the parent is either diagnosed with kidney disease after having children or has a milder phenotype than the affected child. There can also be so-called “survivor guilt” in the unaffected siblings or other relatives who do not carry the mutation(s). The psychological ramifications should be taken into account when counseling a patient or family regarding genetic testing.

Genetic testing may be a useful component of the clinical evaluation of patients with FSGS and nephrotic syndrome. Like any other clinical test, genetic testing must be utilized thoughtfully with considerations of its risks and benefits, and understating of its limitations. New sequencing technologies will continue to rapidly evolve and the cost of genetic testing will continue to fall. Considering that, in contrast to most tests in nephrology, a definitive genetic test result will not change with time (i.e. an ANCA titer or kidney ultrasound may change, but a germline podocin mutation will not), the cost of a clinically useful test is not high. Genetic analysis in the research setting continues to have enormous potential in advancing our knowledge of the biology of disease. Continuing to define relationships between genotype-phenotype correlation studies will fine tune our understanding of the implications of known FSGS genes and improve our understanding of genetic diagnosis.

New technologies

On the surface, it seems “obvious” that it is better to sequence all genes (or at least all coding sequence – the exome) or even the whole genome, than test a patient’s DNA sample for either specific pathogenic mutations or mutations in a specific list of genes. Increasingly, this approach is being implemented not just in the research lab but in the clinic as well. As an intermediate approach between Sanger sequencing and exome or genome sequencing, targeted sequencing using of panels of genes is increasingly being implemented, in which a specific set of genes of interest is captured or amplified from a genomic DNA sample, then sequenced using next generation technologies⁶⁵.

There are certain clear theoretical and practical advantages of employing this sort of approach. A clinician may ask:

1. Why pay a clinical lab thousands of dollars to sequence a few known disease genes when for a thousand dollars or so, a whole exome can be sequenced?
2. Maybe my patient has a mutation in a gene I am not thinking of because I don’t have the diagnosis quite right – perhaps my patient has FSGS secondary to Alport syndrome or nephronophthisis, a diagnosis I could make if I sequence type 4 collagen genes or ciliary protein genes.
3. Maybe I will learn other valuable things about my patient as well from all the other genes in the genome.

Issues in the technical accuracy of sequencing, issues in sequence data interpretation, and issues in clinical interpretation of sequence data make these answers less straightforward than they may seem at first glance ⁶⁶.

Technical accuracy

Next generation sequencing methodologies involve obtaining multiple short reads of sequence from all components of a “library” that may be created using an individual's entire genomic DNA, or DNA from which the exome (or some other subset of genes) has been “captured.” The number of reads of a particular segment of DNA is referred to as its “coverage”. The different available technologies produce somewhat different sets of potential artifacts. Wide differences in coverage between different amplicons affect the confidence with which a specific DNA variant can be called. Missing a heterozygous change is more likely than missing a homozygous change.

Different companies have developed competing platforms for whole exome and whole genome sequencing, as well as different methods and reagents for “capturing” the subset of the genome to be sequenced (exome or other subset). These methods differ in speed, accuracy, and read length^{67, 68}.

Similarly, different software platforms exist for processing this data. Since human beings cannot go through these large data sets, they need to be computationally “filtered” well before a researcher or clinician can reasonably begin to sift through the data resulting from a patient's (or subject's) DNA sequence. Issues facing researchers may be quite different from the issues of a clinician taking care of an individual patient. For example, a researcher may be more interested in whether variants in a gene or set of genes is more common in a group of people with a specific phenotype compared with controls. A clinician needs definite information about the presence or absence of variants in a specific patient. These different needs can influence which sorts of errors (and rates of errors) are tolerable and which are not. Further, more technical discussion of the issues surrounding the use of these methodologies and their application to clinical diagnosis are available in many recent reviews, including references ^{69 and 70}.

Data interpretation

Once a set of DNA variants is identified, the issue of interpretation remains significant. Some of these issues are common to all forms of testing, some are unique to the newer technologies. In the evaluation of a family in which multiple individuals are affected with, say FSGS or SRNS, we can look for cosegregation of disease with genotype. If a mutation that has been previously and convincingly identified as disease causing, say in *TRPC6*, has been identified in a new family with autosomal dominant FSGS, we can conclude with strong confidence that it is disease causing here. If a variant never before observed in *TRPC6* is observed in our patient, then interpretation is more involved. Does the putative mutation segregate with disease in the family? Has it ever been observed before in normal individuals (there are large databases, such as the 1000Genomes Project that can be queried. In the case of a recessive disease, a variant could still be present at non-zero frequency in the population and be disease causing. Many recessive disease alleles (such as F508 in *CFTR*,

or p.R229Q in podocin) are present at reasonably high frequencies in the heterozygous state in most populations. Software programs such as SIFT and Polyphen that try to predict the likely pathogenicity of amino acid changes are useful but imperfect aids to assessing specific variants identified^{71, 72}.

What is the prior probability that our patient has a highly penetrant form of FSGS/NS? The likelihood that a variant in a known FSGS is clinically and pathophysiologically significant is much greater if found in a patient with familial FSGS than in an individual with no history of kidney disease. A recent study by Flannick et al provides a cautionary example⁷³. A non-trivial number of individuals in the general population have what appear to be disease-causing MODY (Maturity Onset Diabetes of the Young) mutations but have no evidence of abnormal glucose homeostasis. This problem is magnified when we move from testing a specific gene set to the set of all genes. We may be able to interpret the presence of homozygosity for a truncating mutation in the nephrin gene *NPHS1* in a nephrotic patient, but how do we interpret the presence of, say, heterozygosity for a rare variant in a recessive nephronophthisis gene in a patient with FSGS? Filtering out low frequency variants and potential artifacts by computational means may reduce the sensitivity of our analysis by preemptively throwing out true disease-contributing alleles. Conversely, too lenient a filter may lead to overcalling many variants with no relevance to disease. Some of the technical issues will improve quickly. Sequencing a panel of genes rather than the entire exome or genome allows the clinician or researcher to focus on reasonable clinical hypotheses, making a difficult problem in data interpretation and analysis somewhat less complex, less time consuming, and less costly. DNA sequencing technology is advancing at such a rapid pace that some of the technical problems noted above may be gone soon after (or before!) this article appears in print. The interpretation issues will remain with us for a longer time.

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Table

Gene	Locus	Inheritance	Protein	Phenotype
Slit diaphragm				
<i>NPHS1</i>	19q13.1	AR	Nephrin	Congenital nephrotic syndrome
<i>NPHS2</i>	1q25.2	AR	Podocin	Congenital, infant, childhood onset SRNS. Also can have adolescent and adult onset SRNS in individuals who carry the common p.R229Q polymorphism
<i>PLCE1</i>	10q23.33	AR	Phospholipase C epsilon 1	Early onset DMS. Some responsiveness to immunosuppression in case reports
<i>CD2AP</i>	6p12	AD	CD2 associated protein	FSGS. CD2AP deficient mouse model develops severe proteinuria
<i>TRPC6</i>	11q22.1	AD	TRPC6	Adult onset FSGS
Actin cytoskeleton				
<i>ACTN4</i>	19q13	AD	Alpha-actinin 4	Adult onset FSGS with incomplete penetrance
<i>INF2</i>	14q32.33	AD	Inverted formin 2	Adult onset FSGS with incomplete penetrance
<i>MYO1E</i>	15q22.2	AR	Myosin 1E	Childhood onset SRNS
<i>ARHGAP24</i>	4q22.1	AD	Arhgap24 (RhoGAP)	Adolescent onset FSGS
<i>ARHGDI1</i>	17q25.3	AR	<u>Arhgdia</u>	Childhood onset SRNS
Nuclear				
<i>WT1</i>	11p13	AD	Wilms' tumor 1	Isolated nephrotic syndrome or as part of Frasier or Denys Drash syndrome
Glomerular basement membrane				
<i>LAMB2</i>	3p21	AR	Laminin beta-2	Isolated nephrotic syndrome or as part of Pierson syndrome
Other				
<i>APOLI1</i>	22q13.1	Recessive risk inheritance	Apolipoprotein 1	Risk haplotypes associated with increased risk of FSGS and ESKD in African Americans
<i>CUBN</i>	10p13	AR	Cubilin	Childhood onset SRNS
<i>NEIL1</i>	15q24.2	AR	Nei endonuclease VIII-like 1 (E. coli)	Childhood onset SRNS

Selected list of genetic causes of non-syndromic nephrotic syndrome and FSGS. AD = autosomal dominant, AR = autosomal recessive. DMS = diffuse mesangial sclerosis.