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Opportunities and challenges of genotyping of patients with nephrotic syndrome in the genomic era

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Abstract

Both targeted and genome-wide linkage and association studies have identified a number of genes and genetic variants associated with nephrotic syndrome (NS). Genotype-phenotype studies of subjects with these variants have identified correlations of clear clinical significance. This, combined with improved genomic technologies, has resulted in increasing, and justifiable, enthusiasm for incorporating our patients' genomic information into our clinical management decisions. Here, we will summarize our understanding of NS associated genetic factors, namely rare causal mutations or common risk alleles in apolipoprotein L1. We then discuss the complexities inherent in trying to ascribe risk or causality to these variants, particularly as we seek to extend genetic testing to a broader group of patients, including many with sporadic disease. Overall, the thoughtful application and interpretation of these genetic tests will maximize the benefits to our patients with NS in the form of more precise clinical care.

Keywords

podocyte; focal segmental glomerulosclerosis; SRNS; risk allele

Introduction

The notion of "Precision Medicine" holds that identifying molecular mechanisms underlying human disease will result in an ability to guide therapy, tailored to a patient's disease signature, in a way that will improve their clinical care and long-term health[1]. This approach has gained traction recently because of a confluence of technical advances[2], the ongoing need for better ways to treat diseases, and high-profile publicity, including the 2015 State of

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the Union Address[3]. Here we discuss a major aspect of "precision nephrology", namely the genetic analysis of individuals with nephrotic syndrome (and related glomerular syndromes and forms of glomerular injury).

Both targeted and genome-wide linkage and association studies have identified a number of nephrotic syndrome and FSGS (NS, FSGS) associated genes and genetic variants.[4,5] Experimental follow-up of these genes and their variants have elucidated key biological mechanisms driving NS.[6] Genotype-phenotype studies of subjects with NS have identified correlations of clear clinical significance.[7–9] As a result, there is increasing, and justifiable, enthusiasm in extending the benefits of these genomic discoveries to as broad of a group of patients as possible. At the same, like any clinical test, there are a number of important factors to consider both prior to doing genetic testing and in interpreting the results. This includes characteristics of the subjects being analyzed, variants being found, and the communication of results to patients, their physicians, or in the literature. Recognizing these strengths and limitations will maximize the benefits of applied genomic medicine to individuals with NS.

Below, we give an overview of the genes and gene variants that have been found to be associated with NS and the major biological and clinical insights resulting from their discovery. We provide some examples that illustrate a number of the complexities inherent in trying to ascribe risk or causality to variants found in sporadically affected subjects with NS. The scope of this review is intended for the general reader without delving too deeply into the technical and methodological aspects of sequencing technologies and statistical genetics.

The spectrum of human genetic variation

In reviewing genetic factors contributing to NS, it is important to first consider some general principles related to human disease-associated genetic variation. Basic concepts of allele frequency, models of inheritance, and penetrance, inform our decisions when considering genotyping our patients. This knowledge also aids in interpreting results.

It is common to think of disease and trait-modifying genetic variants as a function of two parameters, allele frequency and effect size,[10] each of which range continuously from small to large. Individually rare variants with large effect sizes and complete penetrance cause highly heritable, Mendelian disease. The high penetrance means that if a person has the genetic variant, then they will likely exhibit the disease phenotype. Penetrance is typically quantified on a scale of 0 to 1 (or 0 to 100%). Variants that are highly penetrant and very rare are generally referred to as "mutations." On the other hand, genetic variants that are associated with complex diseases or traits and are common in the population typically have relatively modest effects on the phenotype. Rare variants with weak effect are largely irrelevant, and highly penetrant variants do not become common because they undergo negative selection due to their deleterious effect.

SRNS due to rare variants with high penetrance

There are now more than 20 genes reported to harbor mutations that are sufficient alone to cause steroid resistant nephrotic syndrome (SRNS) (Table 1).[4,11,12] These mutation-harboring genes are also termed monogenic or single gene-cause of SRNS.

Meaningful clinical correlates of monogenic forms of NS

There are a number of clinically meaningful phenotypic correlates that have been reported for those with monogenic forms of SRNS. For one, a number of mutations that cause SRNS also result in extrarenal phenotypes. Examples include eye abnormalities in NS caused by laminin beta 2 (*LAMB2)* mutations,[13] Denys-Drash Syndrome or Frasier syndrome in patients with Wilms Tumor 1 (*WT1)* mutations,[14,15] and nail patella syndrome in those with LIM homeobox transcription factor 1, beta (*LMX1B)* mutations.[16] Thus, identifying subjects whose NS is caused by these mutations can thus alert clinicians to screen for other abnormalities.

Sequencing studies of subjects with steroid sensitive NS have not found mutations in known SRNS genes[17,18]. Subjects with NS attributed to a monogenic cause are resistant to immunotherapy.[7,8,12] This has been most well studied in regards to the inability of corticosteroids and/or cyclosporine to achieve remission. Based on these observations of ineffectiveness of immunosuppression, a number of publications suggest that SRNS subjects diagnosed with monogenic NS should perhaps have immunosuppressant medicines withdrawn[8], or at least not intensified[19]. Finally, studies of patients with monogenic NS who have received a kidney transplant have demonstrated that these subjects are at much less risk for recurrent NS in their allograft as compared to those without a known monogenic cause of NS[20].

NS associated with common genetic risk variants

In recent years, genome-wide association studies (GWAS) have identified common genetic risk loci associated with relatively common or complex traits or diseases.[21] Initial association studies genotyped single nucleotide polymorphisms (“SNPs”) to seek trait association. More recently, especially with the advent of next generation sequencing, differing strategies are being used to also identify less frequent or rare variants associated with disease[22–24].

Common SNPs (that are present in healthy members of the population) have also been identified that are associated with increased risk of NS and other glomerular phenotypes[5,25–27]. By genotyping relatively small, but phenotypically homogenous groups of sporadically affected subjects of the same ancestry, risk variants have been found that are associated with proteinuric renal disease in general[28], membranous glomerulonephritis[26], IgA nephropathy[27,29], steroid sensitive nephrotic syndrome in children[25], and FSGS in those of recent African descent[5]. And as opposed to odds ratios conferred by SNPs in complex phenotypes like body mass index[30] or type 2 diabetes[31], the odds ratios for NS related SNPs can be very large. In European individuals homozygous for the two known risk alleles linked to the genes *PLA2R1* and *HLA-DQA1*, the OR for MN is 78[26]. More recently, another

common variant within the *HLA-DQA1* gene (which results in an amino acid change), was found to be associated with a 2.1 increased odds of steroid sensitive nephrotic syndrome in children of both South Asian and European origin[25].

About 12% of all African-Americans have an APOL1 genotype that confers high risk of kidney disease (i.e. both alleles contain risk variants). There are two independent APOL1 alleles, commonly referred to as G1 and G2, that both change the coding sequence of the APOL1 protein.[5] These individuals have a 10–20 times increased odds of FSGS and 30 times increased risk of HIV associated nephropathy if they have HIV[32].

Stated differently, the high-risk *APOL1* genotype is present in about 70–75% of African-Americans with FSGS. Furthermore, among African-American subjects with the same renal phenotype (FSGS, MN, lupus nephritis, CKD), those with the high-risk *APOL1* genotype have more aggressive forms of disease, as evidenced both through clinical parameters and histologic changes[33–35]. Finally, renal allografts from deceased donors with the high-risk *APOL1* genotype have worse outcomes than those from donors without this genotype.[36] However, renal allografts of any origin do not have worse outcomes if they are transplanted into recipients with the high-risk genotype.[37,38]

Genotyping NS Patients

Thus, in terms of currently known NS-associated genetic risk factors (FSGS or minimal change disease), affected patients may harbor rare mutations in previously implicated genes for SRNS or common genetic variants in *APOL1*. The technological capability exists to allow each of these to be genotyped for patients seen in clinic. Occasionally, a patient's insurance company will cover the cost of this test (for example, see http://www.aetna.com/cpb/medical/data/100_199/0140.html).

The major factors that enter into a clinician's decision to perform testing for genetic susceptibility or causation of NS should not differ greatly from making the decision to perform any clinically relevant test. When added to the clinical, laboratory, and/or histological data already obtained, genotyping results should allow the clinician to refine their diagnostic understanding of a patients NS in a way that will result in significant improvements in a patient's care.[39] This could be in regards to a more accurate and prognosis or family counseling for a patient or optimized clinical decision-making in terms of the choice of therapeutics, their dosage, or the appropriate frequency or types of clinical monitoring.

In this current genomic era, our ability to sequence increasing numbers of genes in increasing numbers of NS patients has grown by leaps and bounds[40]. As such, there is an increasing likelihood that the genes and patients genotyped, and variants considered to be mutations (at least in the research arena), will diverge rapidly from those originally studied and published. As practicing nephrologists in the United States, we are particularly interested in appraising genotyping efforts in the sporadically affected adults and children who comprise the vast majority of NS patients for whom we care in our clinics.

As with clinicians and investigators who study causal variation in other human disease,[41] those studying NS also recognize the complexities that arise when trying to attribute causality to rare genetic variants found in affected NS patients[11,42]. And in terms of *APOL1* related kidney disease, how do we (or should we) communicate the concept of risk to patients or family members of a patient with these incompletely penetrant risk alleles?

Attributing causality to rare variants

In considering performing targeted sequencing for monogenic NS in our patients or research subjects, we must be confident in our ability to be accurate in implicating variants as pathogenic and highly confident in our ability to predict the clinical consequences for our patients in whom we find them. Below we will consider a number of major challenges that arise when considering the role of monogenic disease in sporadically affected NS patients. These include (1) generalizing previously published prevalence estimates and clinical correlations for monogenic NS to our population, (2) classifying rare protein-altering variants as pathogenic in the absence of strong genetic evidence, and (3) appreciating the potential for incomplete penetrance of the NS phenotype in subjects with a putative monogenic mutation.

Characteristics of subjects in whom monogenic mutations were originally discovered

The discovery of the vast majority of known monogenic causes of SRNS (including all of the most prevalent) was made possible through the use of family-based genetic discovery methods known as linkage analysis.[43] The success of this approach was predicated upon the genotyping of numerous independent large families with multiply affected members and identifying regions of the genome segregating with the disease phenotype. Performing discovery research in consanguineous families has been particularly effective in increasing the statistical power to detect causative mutations, because affected offspring can be assumed to have inherited the disease-causing variant from a common ancestor. Finally, as in most Mendelian traits, their prevalence is enriched in diseases of pediatric onset.

Characteristics of the variants that built genetic evidence to implicate them as causal mutations

In addition to studying the optimal subjects, numerous complementary lines of statistical and functional evidence have been employed to build strong evidence of causality of these mutations for SRNS. Stringent statistical thresholds for linkage analysis are one of the initial steps used to eliminate false positive loci. The interpretation of loss of function (LOF) variants (e.g truncating or splice site), that result in absence or severely abnormal forms of the protein tend to be easier to interpret: an absent protein cannot be functional, while it is harder to know the effect of a missense mutation. Within a family, if a gene variant does not segregate with disease under some plausible model of inheritance, then it cannot be considered causal. The identification of independent families with independent mutations strengthens the case that a set of mutations in a gene are in fact causal. And finally, experimental models systems have been used to demonstrate that disruption of the candidate gene resulted in an NS phenotype. This combination of genetic and functional information, when present, provides the most compelling case that a gene is disease-causing. Not

infrequently, however, the relationship between a gene and its variants to disease cannot be proven unequivocally.

Prevalence reports of monogenic NS

Thus, using very stringent statistical and functional criteria in rare and highly informative affected families, a group of single-gene causes of NS have been discovered. Over the years, the coding sequence of these genes have been sequenced in diverse groups of subjects with NS, increasing our understanding of the spectrum of disease associated with alterations in specific genes. The prevalence estimates for monogenic causes in people with SRNS vary substantially between studies, ranging from <1% to 33%[12,44–49]. This variation is due to a number of factors.

In a cohort of Finnish subjects with congenital nephrotic syndrome (CNS), positional cloning efforts identified nephrin defects as causal; mutations in the nephrin gene (*NPHS1)* were identified in 94% of affected subjects[50]. Sequencing of *NPHS1, NPHS2, LAMB2,* and *WT1* in 46 families of European and Turkish origin with CNS found a prevalence of monogenic disease in 91% and 64% in families, respectively[51]. Two recent studies that sequenced panels of genes in adolescents with SRNS reported a prevalence of monogenic disease of approximately 11%[12,52]. In a single study of European adults with end stage renal disease (ESRD) secondary to FSGS, 8% were attributed to mutations in one of seven genes.[53] In another European sequencing study of eight genes in adults with SRNS, the monogenic prevalence was 14%.[54] By contrast, similar sequencing studies that applied the same filtering criteria in subjects with SSNS reported a 0% prevalence of monogenic disease[12,17,18].

Finally, a significant direct association is observed between the prevalence of monogenic NS and consanguinity. For example, in a recent study of worldwide SRNS subjects <25 years of age, those from the United States, in which consanguineous unions were absent, the prevalence of monogenic disease was about 13%[12]. This contrasted with a rate of 45% at the location with the greatest degree of consanguinity.

Altogether, these data consistently report a higher prevalence of monogenic NS in those with early-onset NS (particularly congenital NS), a family history of disease, and those from consanguineous unions[42,55]. This information is critical in regards to estimating the true (or expected) prevalence of monogenic disease in a patient or population who is undergoing sequencing and analysis. For one, the estimated chance of getting a positive result will influence the decision by a clinician to perform genetic testing. Secondly, the expected prevalence of bona fide monogenic disease in a subject (i.e. the pretest probability) will aid in interpreting whether a positive screening test is truly accurate. We expand on this below.

A closer study of nephrin (NPHS1) as single-gene, recessively transmitted cause of NS

Perhaps the most phenotypically severe inherited glomerulopathy is congenital nephrotic syndrome (CNS) caused by mutations in both *NPHS1* (nephrin) alleles.[50] It is now clearly established that absence of functional nephrin causes CNS and that a large percentage of infants, particularly of Finnish or Northern European ancestry have causative mutations in

this gene. Nephrin is a large integral membrane protein that appears to have structural as well as signaling functions.[10] Between 1–2% of SRNS in children greater than age 1 is attributed to *NPHS1* mutations[12,54]. Thus, we would not be surprised if an infant with severe CNS underwent targeted genotyping of *NPHS1* and two LOF alleles ("Fin-major") were identified. In other words, we are comfortable attributing causality of the homozygous Fin-major allele to an infant's CNS and using published phenotypic correlates of this genotype to guide our clinical care.

In the current era, the *NPHS1* gene can be clinically sequenced individually and is also typically included on research and clinically available diagnostic panels of known monogenic NS genes. Thus, there is the increasing probability that in addition to infants with CNS, *NPHS1* sequencing will now be performed subjects whose phenotype do not closely match those subjects in which the initial discoveries were made. For example, how do we interpret the genotype of an infant with CNS from Finland (where CNF is relatively common), in whom heterozygosity (rather than homozygosity) for Fin-major and no other *NPHS1* variant is detected? The prior probability that this infant has *NPHS1*-associated NS is so high that we might question the accuracy of the genetic analysis. In fact, it would not be uncommon in this situation to reanalyze or even resequence *NPHS1* in this patient with the belief that the second mutation was missed. By contrast, if we were to analyze the *NPHS1* sequence of a 60 year old man in good health and no microalbuminuria, the presence of two putative loss of function mutations would lead us to conclude that either there was an error in genotyping, or that one or both of these genetic variants is in fact relatively benign.

In both of these cases, one could argue that the very high and very low prior probability of *NPHS1*-associated disease makes genotyping relatively uninformative, at least for diagnostic purposes. These two examples represent the extremes in terms of patient age, predicted severity of mutation, and clinical phenotype. A more challenging example would be how to interpret the results of *NPHS1* sequencing in a sporadically affected American child or adult presenting to the nephrology clinic for the first episode of NS prior to receiving therapy. Or interpreting *NPHS1* sequencing results in the same patient who has not yet responded to 6 weeks of appropriate corticosteroid therapy. In both of these situations, the prior probability of an affected subject having a monogenic cause of NS due to *NPHS1* mutations is small, but not negligible. Below, we will discuss how our ability to classify the pathogenicity of rare variants and incomplete penetrance affects our ability to predict clinical outcomes for patients harboring putative mutations in known SRNS genes.

Challenges in accurately classifying the pathogenicity of variants

As opposed to LOF variants, whose functional consequences seem much more certain (although exceptions to this also apply)[56], attributing causality to missense variants relies primarily on predictions based on allele frequency thresholds in the population, measures of nucleotide conservation across species, and functional prediction software. For most missense variants identified, functional testing of their pathogenicity in model systems has not been performed and is clearly not practical for every new missense variant identified. To further complicate matters, for many inherited disorders, particularly those inherited in a

dominant manner, LOF alleles may not be disease causing[57] whereas specific subsets of missense changes, causing specific functional changes, may be. This certainly appears to be the case with dominant forms of FSGS caused by mutations in *ACTN4*,[15] *TRPC6*,[10] and *INF2*.[18]

The complexity is significant in attempting to attribute causality to a variant found in targeted sequencing studies. For clinically certified sequencing laboratories whose ordering physicians may act on these tests, the stakes may be very high. As a result, clinical labs set a high bar in calling variants "disease-causing." More often then not, such results are reported as inconclusive, and variants are labeled as "of unknown significance."

However, in the research setting, the stringency of attribution of pathogenicity to variants found in next generation sequencing is more variable. This is especially pertinent when trying to interpret the pathogenicity of missense variants, which are not as clearly damaging as LoF variants. To begin, the amount of sequencing performed for each gene and the parameters used for low level processing and variant calling of the next-generation sequencing is not yet standard. These factors introduce variability even prior to beginning the filtering of a set of variants for possible pathogenicity. Different, and somewhat arbitrary, criteria for phenotype classification can alter the interpretation of such variants. In terms of *in silico* pathogenicity filters, investigators may use different maximum thresholds for the frequency in which a candidate variant can be present in a control population. They may also use different approaches to account for genetic differences resulting from ancestry differences. Different levels of conservation of an allele across species may be specified and different individual or combinations of functional prediction software may be employed.

Taken altogether, these factors can result in variable prevalence estimates of monogenic disease in a population and potentially inaccurate inferences regarding phenotypic correlates to monogenic disease (Table 3). This is a critical issue that is generalizable to all human disease research surrounding causal sequence variants. As stated in a 2014 Perspectives article in Nature by MacArthur et al, "The discovery of rare genetic variants is accelerating, and clear guidelines for distinguishing disease-causing sequence variants from the many potentially functional variants present in any human genome are urgently needed. Without rigorous standards we risk an acceleration of false-positive reports of causality, which would impede the translation of genomic research findings into the clinical diagnostic setting and hinder biological understanding of disease."[41]

Variable expressivity within known monogenic genes

Despite the challenges articulated above, most research teams use a set of filters that, in combination, result in a set of variants that by most accepted measures of frequency, conservation, and affect on the protein, would be considered as pathogenic and causal for SRNS. Yet even then, the presence of a single putative pathogenic mutation in a dominant NS gene or two mutations in a recessive NS gene does not guarantee that a patient will have or will develop SRNS.

Subjects with putatively pathogenic mutations in SRNS genes at times have variability in disease expression[20,55,56,59–63]. In some cases, a subject with monogenic NS appears to be

sensitive to immunotherapy or does not have rapid renal functional decline. In other situations, siblings or parents of a child with monogenic SRNS are asymptomatic despite having the same mutation.

In considering these cases, it is certainly possible that a subject or family member in fact will develop SRNS that had not yet manifested. But other possibilities exist. It may be that additional genetic or environmental factors may determine if the disease manifests at all, or affects a subject's response to therapy or degree of renal functional impairment. Or the specific mutations within a gene may affect expressivity. A 2014 study on variant specific pathogenicity in *NPHS2* demonstrated that for individuals with SRNS, having two predicted pathogenic mutations (one on each chromosome) in the recessive disease gene *NPHS2* does not always result in monogenic SRNS.[58] They showed statistically and functionally that a common NS-associated variant, pArg229Gln of *NPHS2* is only disease causing when there are very specific subtypes of pathogenic variants on the other allele.

In general, the true penetrance of a variant or set of variants in a gene are likely less than that suggested by initial gene identification studies[64,65]. Initial gene discovery efforts are performed using family-based approaches in clear familial cases of disease. Clinicians will not recognize those families in which low penetrance masks the inherited nature of a phenotype. Together, these factors tend to overestimate the penetrance associated with variants in a specific gene.

Thus, based on what is known from family-based genetic studies, it may be hard to extend conclusions from family based studies to sporadically affected patients as to the extent to which a specific variant in, say, *INF2*, *TRPC6*, or *ACTN4*, is causing or contributing to NS. It is even more complicated for variants that have never observed before and which have never studied biochemically or tested in an animal model.

Another possibility is that, despite their prediction, a proportion of rare, predicted protein-altering variants in known monogenic SRNS genes in fact have no functional consequence at all. In this scenario, the subject's NS would be completely unrelated to these variants observed. Large new publicly databases of genetic variants in control individuals (or individuals included without regard to phenotype) provide a wealth of data that allow investigators or clinicians to determine how often a given variant is seen in the general population (e.g. the ExAc browser, at http://exac.broadinstitute.org/). From analysis of exome sequencing across thousands of healthy controls and subjects with a variety of diseases, we now recognize that there is a non-trivial amount of (individually) rare variation, often of predicted negative consequence, in all human genomes.[57,66,67] This includes both missense variants as well as clear LoF variants. In other diseases, the prevalence of rare, putatively harmful variants in genes known to harbor causal mutations observed in healthy controls is far greater than the reported prevalence of the disease.[68,69] A quick perusal of the publicly available databases of genetic variants shows that both common variants and rare missense variants, predicted to be deleterious, are present in most of the known FSGS/NS genes, for example, rare *INF2* variants in the 1000 Genomes Project subjects (Table 2).

Incorporating these insights into clinical decision making both whether to order genetic testing or interpret test results

This becomes relevant in the current era in which increasing numbers of patients may undergo sequencing due to decreases in cost of the technology and/or increased enthusiasm for the benefits of "precision medicine." It is now possible for subjects at initial presentation, or those with steroid sensitive NS (infrequently relapsing, frequently relapsing, or steroid dependent) to undergo diagnostic sequencing. If a putative pathogenic mutation is found in these scenarios, what is a clinician to do?

As previously discussed, a review of the existing literature suggests that if an NS subject has a clear causal mutation, then he or she will be resistant to immunotherapy, but that if they have SSNS, they will not have pathogenic mutations. However, given the rarity of these mutations, or the stringency of the filters employed, and the likely existence of inflammatory stimuli that interact with genetic susceptibilities, it seems likely that future studies may identify genetic forms of FSGS/NS that show response to steroids and other immunosuppressive therapies. Regardless, we predict that as we expand our scope of subjects studied, we will increasingly detect rare variants in the genomes of subjects with NS which are nonpathogenic, even though they have been previously reported as harmful or are predicted as such by conventional. Maximizing our ability to recognize this group of putatively pathogenic variants that have no functional consequence, or situations in which they may have variable expressivity, is critical to prevent clinical decisions to be made based on inaccurate classification or prediction.

The complexity of genome-wide, rather than targeted sequencing

The ability to perform whole exome sequencing, generating sequence data on 20,000 genes, complicates genetic analyses even further. We may perform exome sequencing with a particular interest in a specific gene or set of genes from a biologic pathway. But since we have performed an analysis of ~20,000 genes (and hypotheses), we need to set very stringent statistical thresholds to establish statistical support for pathogenicity of any variants.

Another complication comes from performing whole exome (or even whole-genome) sequencing in individuals with sporadic (non-familial) FSGS. In these studies, it is much more likely than not that we will not obtain a clear causal genetic alteration. At times, we will find no deleterious variant in any previously described NS/FSGS gene, but do observe a genotype widely considered to be a cause of other renal disorders like nephronophthisis[70] or Alport syndrome[71]. Do we conclude that our patient has been misdiagnosed? Or that mutations in these genes are also causes of FSGS?

In consideration of the APOL1 risk genotype

The challenges of sequencing sporadic NS subjects to determine if they have rare and causal NS mutations include issues surrounding quality of next-generation sequencing, attributing pathogenicity to variants discovered, and incomplete penetrance. By contrast, the challenges with the relatively common *APOL1* risk alleles are different and relate to communicating and understanding risk as well the relative infancy of this area of inquiry.

While on the one hand, the high-risk *APOL1* genotype is not sufficient to cause disease, the increased risk associated with this genotype is very high.[21] Thus, it is reasonable to infer that these risk alleles represent a causal contribution to disease susceptibility. While we do not currently suggest routinely performing *APOL1* genotyping in a clinical setting, we suspect that future studies will show that clinical screening of *APOL1* in certain subgroups of African Americans with kidney disease will be beneficial.

The NEPTUNE study as a laboratory for genotyping in sporadic NS subjects

Fundamentally, in regards to diagnosing monogenic forms of NS in the genomic era, we are challenged by the goal of wanting to make specific, personalized, genetic diagnoses in each individual with disease while at the same time recognizing current limitations in perfectly classifying the pathogenicity of variants and distinguishing a specific variant's degree of causality versus susceptibility. This is particularly relevant to the care of patients in places such as the United States, where the affected subjects are mostly sporadically affected and of diverse ancestries. There is a clear need for population-based studies to help improve our ability to classify variants and to understand issues of causality versus association. The Nephrotic Syndrome Study Network (NEPTUNE), a prospective, observational study recruiting a cohort of North American subjects from adult and pediatric nephrology clinics presenting with proteinuric primary glomerular disease who need a clinically indicated renal biopsy, presents an opportunity to study these issues.[72]

Based on summary statistics of 455 subjects recruited in NEPTUNE, the mean age (interquartile range) of subjects is 34 (37.5). There is a diversity of races, including 24% black, 55% white, and 12% Asian. Histologic diagnosis shows that 26% have MCD, 32% have FSGS, 15% with MN, and 27% with other glomerulopathies. While recruited near initial presentation of NS, a number of subjects have already received immunosuppression. However, response to immunosuppression is not an enrollment criteria. At recruitment, each subject has blood, urine, and renal biopsy material obtained for intrarenal gene expression data generation. Each subject is regularly followed for at least 5 years with follow up clinical and biochemical data collected. From a genetic perspective, each subject is undergoing targeted next-generation sequencing of 20 known monogenic SRNS genes, *APOL1* genotyping, Exome Chip genotyping, and low-depth whole genome sequencing.

Determining the prevalence of monogenic and *APOL1* associated forms of NS in the NEPTUNE cohort will differ significantly from many previous genetic studies of NS. The inclusion criteria for NEPTUNE is quite broad and thus there is limited ascertainment bias toward enrichment of monogenic disease (e.g. pediatric onset, SRNS or FSGS). Other unique aspects of genetic analysis in this cohort include its prospective nature and generation of paired genomic datasets. A challenge in genetic analysis is that NEPTUNE is not currently genotyping the parents of enrolled subjects, which may reflect the scenario in other research studies or clinical care.

Altogether, NEPTUNE aims to determine the prevalence of predicted monogenic NS in a less-selected cohort that closely reflects the population cared for in the United States and to determine the phenotypic correlates of those with putative monogenic mutations. At the same time, NEPTUNE hopes to improve the ability to predict the pathogenicity of variants

of unknown significance. And finally, in terms of *APOL1*-related NS, the project will seek to understand if the high-risk genotype impacts outcomes independent of classical histologic diagnosis. Intrarenal gene expression data will be used to discover molecular signatures that are unique to those with the high-risk genotype and which may illuminate the biological mechanisms that mediate this disease.

Conclusions

As with any clinical test, it is important for clinicians to consider why a test is being ordered, whether or not it will affect diagnosis or clinical care, or whether prior probabilities render a test result essentially meaningless. Despite the complexities in interpretation of genetic analyses in individuals with nephritic syndrome, there seem to be specific scenarios in which there is benefit in screening for rare and highly penetrant mutations. As we have discussed above, for the infant with congenital nephrotic syndrome, identifying mutations in *WT1* or *LAMB2* would dictate further screening for extra-renal phenotypes, while identifying *NPHS2* or *NPHS1* mutations would remove concern for extra-renal manifestations. Identifying known mutations in patients with the NS phenotype could also help with genetic counseling and family planning decisions, although there are challenges inherent in this in regards to the potential for variable expressivity. If a patient is clinically found to be unable to achieve complete remission with steroids, subsequent identification of a mutation in a known SRNS/FSGS may suggest that the patient is unlikely to respond to intensification of steroid therapy. Finally, in the setting of an NS patient needing a kidney transplant, if genetic analysis identifies a bona fide causal mutation in the patient, related family members at risk for disease (based on genetics) can be eliminated as donor candidates.

As both genomic medicine and choices of immunosuppressive therapy expands in NS, we will have to continue to evaluate our ability to classify variants and whether there are generalizable clinical correlates. For example, just because it is well-established that rare mutations in *TRPC6* are a cause of NS and FSGS, it does not mean that the presence of any *TRPC6* variant is in fact the cause of disease in an NS/FSGS patient. Nor does its presence in someone without disease necessarily predict its future development. As genetic analyses become faster, cheaper, and more accessible, it is increasingly important such analyses be applied thoughtfully.

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Table 1

Selected genes harboring causal mutations for steroid resistant nephrotic syndrome

Dominant inheritance	Recessive inheritance
ACTN4	*ADCK4*
ARHGAP24	*ARHGDIA*
CD2AP	*COL4A3*
CFH	*COL4A4*
INF2	*COQ2*
LMX1B	*COQ6*
TRPC6	*EMP2*
WT1	*ITGA3*
	ITGA4
	LAMB2
	MYO1E
	NPHS1
	NPHS2
	PDSS2
	PLCE1
	PTPRO
	SCARB2
	SMARCAL1

Table 2

Predicted harmful variants in *INF2* present in the 2535 subjects of the 1000 Genomes Phase 3.
Qualifying variants had an allele frequency <0.5% in the Exome Variant Server and are either loss of function variants or, if missense, predicted to be damaging by at least two of the following protein prediction programs; SIFT, PolyPhen2, & Mutation Taster. Continental ancestry is the genetically defined ancestry of each subject, as defined in the 1000 Genomes Project.

Continental Ancestry	Subjects affected	Chr	Position	SNP ID	Amino acid change
EUR	1	14	105167973	rs200247054	R91C
SAN	1	14	105172377		L236R
EUR	1	14	105173332	rs145742634	L310Q
AMR	1	14	105174066		P488S
AFR	4	14	105174066		P488S
ASN	10	14	105175646	rs138577569	R660W
AFR	1	14	105175657	rs144069981	D663E
AFR	1	14	105176489	rs371129528	R735W
EUR	1	14	105177495	rs200261709	R797H
AMR	1	14	105178778		L833P
AMR	1	14	105178789	rs201534539	R837C
SAN	1	14	105179616	rs199873407	R950W
EUR	1	14	105179616	rs199873407	R950W
EUR	1	14	105179869		T989I
SAN	1	14	105179881		R993H
AFR	1	14	105180740		D1081Y
AMR	1	14	105181062	rs201715539	S1188F
EUR	1	14	105181173		R1225H
EUR	1	14	105181624		V1233D
ASN	1	14	105181663		C1246S

Chr-Chromosome; ***EUR***-European; ***SAN***-South Asian; ***AMR***-American; ***ASN***-East Asian; ***SAN***-South Asian; ***SNP***-Single nucleotide polymorphism

Table 3

Potential challenges in making the diagnosis of a monogenic form of NS from sequence data

Identifying NS subjects who have bona fide monogenic forms of the condition can improve the precision of clinical management. However, a number of factors contribute to the challenges of both classifying rare variants identified as truly deleterious and, if so, predicting the consequences of their presence.

- Lack of functional testing in model systems of most missense variants
- Imperfect *in silico* pathogenicity pipelines
- Variable expressivity of pathogenic mutations
- Interpreting pathogenicity of variants in NS subjects who are not steroid resistant
- Inheritance of recessive NS only with certain combinations of two pathogenic mutations