Genetics of Nephrotic Syndrome Presenting in Childhood: Core Curriculum 2019

Andreia Watanabe, Luciana S. Feltran,* and Matthew G. Sampson*

Nephrotic syndrome (NS) is one of the most challenging conditions to manage and treat, partly because we lack a specific molecular understanding of its pathogenesis and progression. This limits our ability to provide targeted therapy or precise prognostications. Fortunately, genomic discovery in NS and its translation to genomic-informed medicine is allowing us to improve our understanding of the molecular anatomy of NS and our ability to care for patients with NS. In this Core Curriculum, we review the specific genes and loci discovered in childhood NS, specifically NS of Mendelian origin, *APOL1*-associated NS in black patients, *HLA* region variants associated with steroid-sensitive NS, their biological impacts, prevalence across populations, and clinical correlates. We also review the fundamentals of genetic architecture of human disease, technologies, and analytic strategies that currently exist to discover disease-related genetic variations. A facility with the concepts and vocabulary of modern genomics and ability to interpret results of genetic studies are essential skills for nephrologists caring for children with NS. As such, we hope to empower them to understand the literature in this area, appropriately order genetic tests and accurately interpret the results, and consider how they may participate in or drive the next wave of genomic discoveries in NS.

Complete author and article information provided at the end of the article.

**A.W. and L.S.F. contributed equally to this work.*

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Introduction

Nephrotic syndrome (NS) is one of the most commonly encountered conditions for nephrologists and arguably one of the first topics about which we are expected to become expert. Historically, nephrologists have cared for the children with NS and have discovered a great deal about its pathophysiology, without knowledge of the genomic basis of these conditions. While we have been effective in doing so, we recognize that genomic discovery can increase our knowledge of the molecular basis of NS. Translating these discoveries to genomics-informed care can improve outcomes for these patients.

Genomic Terminology

With increasing proliferation and diversification of clinical genetic testing, it is essential for clinical nephrologists to have a conceptual model and vocabulary surrounding the genomic architecture of kidney diseases. Genetic architecture has been defined as "the landscape of genetic contributions to a given phenotype." One parameter describing this architecture is the type of genetic change, ranging from a single-nucleotide variant (SNV) to insertion or deletion of a small number of bases (indels) to larger structural variants, which can include deletions or duplications (copy number variants [CNVs]) or chromosomal rearrangements (eg, inversions and transversions).

Other parameters include the variant location (coding/noncoding) and frequency in a reference population (ranging from absent to common). Finally, we can describe genetic architecture of a disease based on the effect size of a variant, ranging from alleles that confer low to moderate risk for disease (risk alleles) to those that are fully penetrant (when present, are sufficient to cause a disease: causal variants or mutations).

Terms such as mutations and polymorphisms are often used to describe genetic variants that are either rare (or novel) and fully penetrant or common and harmless, respectively. These are imperfectly accurate terms because many rare variants are harmless, while polymorphisms can increase disease risk. It is less ambiguous to describe a variant as a function of its frequency in a population (common, low frequency, rare, and novel), whether it is coding or noncoding, and its impact on disease (causal/pathogenic, risk, protective, and not associated). There are a number of tools and databases available to help predict the pathogenicity of variants found in genetic studies (Table S1). Table 1 and Box 1 provide a glossary of terms and a guide to nomenclature often encountered in specifying genetic variants.

Additional Readings

- den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS recommendations for the description of sequence variants: 2016 update. *Hum Mutat.* 2016;37(6): 564-569.

The Core Curriculum aims to give trainees in nephrology a strong knowledge base in core topics in the specialty by providing an overview of the topic and citing key references, including the foundational literature that led to current clinical approaches.

Table 1. Frequently Used Nomenclature for Genetic Variation

Term	Definition	Examples[a]	Meaning
Substitution (>)	One nucleotide is replaced by another nucleotide	c.686G>A, p.R229Q	Substitution of guanosine nucleotide by adenosine nucleotide at the indicated position of the coding (c.) DNA, determining a change of arginine (R) to glutamine (Q) at the listed protein (p.) position
Deletion (del)	≥1 nucleotide is not present	c.1106_1111del, p.387_389del	Deletion of 6 base pairs found at the indicated position of the coding DNA, determining the deletion of 2 amino acids in the protein sequence
Insertion (ins)	≥1 nucleotide is inserted	c.3243_3250insG, p.1084fs*12	Insertion of a guanosine nucleotide at the indicated coding DNA position, determining a frameshift and a stop codon after a sequence of 12 amino acids at the listed protein position[b]
Duplication (dup)	A copy of ≥1 nucleotide is inserted directly 3′ from the original copy of that sequence	c.253_264dupGCATACATGTTT, p.Ala85_Phe88dupAYMF	Duplication of 12 base pairs at the indicated coding DNA position, determining duplication of 4 amino acids (alanine, tyrosine, methionine, and phenylalanine) at the listed protein position

[a]The listed examples reflect a substitution in *NPHS2,* the deletion corresponding to the *APOL1* G2 risk allele, an insertion in *NPHS1,* and a duplication in *TRPC6,* respectively.
[b]In other nomenclature schemes, such as that of the Human Genome Variation Society, this would be described as a deletion-insertion (delins).

- MacArthur DG, Manolio TA, Dimmock DP, et al. Guidelines for investigating causality of sequence variants in human disease. *Nature.* 2014;508(7497):469-476. ★ **ESSENTIAL READING**
- 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(5):405-424. ★ **ESSENTIAL READING**
- Timpson NJ, Greenwood CMT, Soranzo N, Lawson DJ, Richards JB. Genetic architecture: the shape of the genetic contribution to human traits and disease. *Nat Rev Genet.* 2018;19(2):110-124.

Mendelian NS Gene Discovery and Clinical Correlates

Case 1: *A 1-week-old full-term white female infant presents with edema, oliguria, and increased serum creatinine. Prenatal and birth history are unremarkable, and there is no family history of kidney disease. During the past 3 days, she became increasingly sleepy and puffy and her parents took her to a local emergency department. There was initially concern about brain hemorrhage or intoxication because the baby had pinpoint pupils, but computed tomography results and toxicology screen were negative. A sepsis workup showed no signs of infection, but was significant for proteinuria (4+), serum albumin level of 2.0 g/dL, and serum creatinine level of 1.7 mg/dL.*

Question 1: You wonder if this patient has a genetic form of NS. Which one of the following statements is correct?

a) Lack of family history argues strongly against a genetic form of NS.
b) Her age of presentation would strongly favor a genetic form of NS.
c) It is not possible to consider a genetic form of NS without knowing whether the disease is resistant to steroids and other immunosuppressive agents.
d) The presence of extrarenal manifestations would exclude a genetic form of NS.

For the answer to the question, see the following text.

The first genes implicated in NS were those harboring exonic variants that were rare or absent in healthy populations and resulted in change in protein function that were sufficient to cause disease. The first of these "Mendelian" (or "monogenic") NS genes, discovered in the 1990s, were Wilms tumor 1 (*WT1*) in Denys-Drash syndrome and nephrin (*NPHS1*) in infants with congenital NS. The statistical power to make these discoveries, particularly for *NPHS1*, was dependent on studying multiple independent families of sufficient size with enough affected individuals. Since then, more than 55 additional genes have been implicated in monogenic forms of NS (Table S2). These discoveries, aided by technological advances in large-scale sequencing, remain reliant on finding families in which the affected patient harbors causal variants that are not found in their healthy family members and are ultrarare or absent in the population. In doing so, it is critical to ascertain informative pedigrees with enough power to identify causal variants from a single or modest number of families that map to the same locus/gene.

Most of the protein products of these monogenic NS genes localize to the podocyte. This discovery was a critical step in identifying this cell as central to the pathogenesis of NS. Functional characterization of these genes has illuminated key biological pathways in the pathogenesis of NS. Among the first discoveries were genes implicated in the maintenance of the slit diaphragm (*NPHS1* and *NPHS2* [encoding podocin]) and regulation of actin cytoskeleton (*ACTN4*). These discoveries have been followed by the discovery of other genes involved in other important processes, such as calcium channel signaling (*TRPC6*), mitochondrial energetics (*COQ2*, *COQ6*, and *PDSS2*), and nucleocytoplasmic transport (*NUP93*, *NUP107*, *NUP205*, and *XPO5*). Importantly, many of these key NS pathways may not have been recognized and studied without being implicated through Mendelian discovery.

From a clinical perspective, there are certain characteristics that are enriched in patients with Mendelian forms of

Box 1. Genetics Glossary

- **Variant**: a difference in DNA sequence compared to a reference DNA sequence
- **SNV (single-nucleotide variant)**: change of a single nucleotide in a DNA sequence
- **SNP (single-nucleotide polymorphism):** similar to SNV, but the variant is common in a population
- **Exonic variant:** variant located in coding region of a gene
- **Missense variant:** SNV that leads to the replacement of the amino acid that would normally be encoded with another amino acid
- **SNP array:** a type of chromosomal microarray in which patient genotypes are determined by hybridizing the patient's DNA to DNA probes corresponding to hundreds of thousands to millions of SNPs
- **indels:** The gain and loss of a small number of bases in a DNA sequence (if exonic and in frame, can alter an amino acid sequence in the encoded protein; if out of frame, it can truncate the amino acid sequence)
- **CNV (copy-number variant):** A structural variant that results in gain or loss of part of a larger number of bases
- **Array CGH (array comparative genome hybridization):** a method to detect deletions and duplications of DNA (eg, for CNV screening)
- **Monogenic/Mendelian disease:** A disease for which the pathogenic consequences on protein function from a rare variant are sufficient to cause a disease
- **Incompletely penetrant:** the proportion of individuals with a certain genetic variant who display the phenotype that is associated with this variant is <100%
- **Risk alleles:** A genetic variant that, when present, increases a person's risk for a specific condition, but is not sufficient to cause disease
- **Modifiers:** A second genetic variant that can modify the expression/function of an initial genetic variant

NS (Box 2). First, they have been discovered almost exclusively in 2 groups; patients (mostly children) with steroid-resistant NS (SRNS; most of whom have focal segmental glomerulosclerosis [FSGS] on biopsy) and infants with congenital NS. In addition, sequencing studies have consistently found that patients with steroid-sensitive NS (SSNS) do not have known monogenic forms of NS.

Many more children with NS have now undergone targeted panel sequencing for a large number of monogenic NS genes through diverse modalities (Table S3). The children undergoing sequencing still mostly have SRNS, but there are some studies that include children with SSNS or NS responsive to other treatment. Although the majority of children with monogenic NS have disease resistant to steroids and other immunosuppressant agents, there are enough reports of children with monogenic NS achieving complete remission to suggest that the drug-resistant phenotype is not absolute. In addition, very recent studies have discovered at least 6 genes in patients with NS that are at least partially responsive to immunosuppressant therapy.

The other major clinical correlate for children with Mendelian NS is a lack of recurrent disease after transplantation. This was noted in the earliest studies of NS due to *NPHS2* causal variants and has since been robustly replicated in larger panels of children with Mendelian genes who have reached end-stage kidney disease (ESKD). This provides important information for clinicians, patients, and families when providing counsel and making decisions about transplantation, particularly choice of donors. For example, if the patient does not have Mendelian disease, the transplantation group should have a higher concern for recurrence. This could also affect the choice of using a living donor and the use/timing of peri-transplantation plasmapheresis and immunosuppression. In addition, potential living related donors of patients with Mendelian disease should undergo targeted screening of the implicated gene to guide decisions about donation.

Although for the most part an isolated condition, Mendelian forms of NS may also be a part of a syndrome that affects multiple organ systems. Examples include Denys-Drash syndrome due to causal variants in *WT1*, Pierson syndrome (*LAMB2*), and nail-patella syndrome (*LMX1B*). More recently, Mendelian causes of NS have been discovered as part of Galloway-Mowat syndrome (genes of the KEOPS complex), a syndrome with skin and lung involvement (*ITGA3*), and a syndrome with ichthyosis, adrenal insufficiency, immunodeficiency, and neurologic deficits (*SGPL1*).

Returning to case 1, the patient with congenital NS and anisocoria, the combination of age of onset and an extrarenal sign increases the likelihood of having a Mendelian form of NS. We would recommend genetic testing in the form of single-gene sequencing of *LAMB2*. Choosing to sequence only 1 gene is sensible because of its high pretest probability. If this patient did not have a causal variant in

Box 2. Clinical Aspects That Suggest a Monogenic Form of Pediatric NS

- Younger age at NS onset (particularly <3 mo)
- Consanguinity
- Familial history of NS
- FSGS or diffuse mesangial sclerosis histology
- Extrarenal abnormalities described as part of syndromic NS
- Resistant to steroids[a]
- No NS recurrence with kidney transplantation

Abbreviations: FSGS, focal segmental glomerulosclerosis; NS, nephrotic syndrome.
[a]Drug-resistant phenotype is not absolute.

LAMB2, we would move to whole-exome sequencing or panel sequencing of the 30 to 50 implicated Mendelian NS genes, depending on costs and/or availability. The correct answer is therefore (b).

Additional Readings

- Boute N, Gribouval O, Roselli S, et al. NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet.* 2000;24(4):349-354.
- Dorval G, Gribouval O, Martinez-Barquero V, et al. Clinical and genetic heterogeneity in familial steroid-sensitive nephrotic syndrome. *Pediatr Nephrol.* 2018;33(3):473-483.
- Gbadegesin R, Hinkes B, Vlangos C, et al. Mutational analysis of NPHS2 and WT1 in frequently relapsing and steroid-dependent nephrotic syndrome. *Pediatr Nephrol.* 2007;22(4):509-513. ★ **ESSENTIAL READING**
- Kestila M, Lenkkeri U, Mannikko M, et al. Positionally cloned gene for a novel glomerular protein–nephrin–is mutated in congenital nephrotic syndrome. *Mol Cell.* 1998;1(4):575-582.
- Machuca E, Benoit G, Antignac C. Genetics of nephrotic syndrome: connecting molecular genetics to podocyte physiology. *Hum Mol Genet.* 2009;18(R2):R185-R194. ★ **ESSENTIAL READING**
- Pelletier J, Bruening W, Kashtan CE, et al. Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. *Cell.* 1991;67(2):437-447.
- Sampson MG, Pollak MR. Opportunities and challenges of genotyping patients with nephrotic syndrome in the genomic era. *Semin Nephrol.* 2015;35(3):212-221.

Prevalence of Genetic Causes of NS

Mendelian Forms of NS

Case 2. *A 14-year-old Brazilian female adolescent who underwent transplantation for kidney failure due to NS is seen in the emergency department for pneumonia. She moved to the United States at 2 years of age and NS was diagnosed at age 4 years. She had no other significant medical history and no family history of kidney disease. The NS was resistant to all immunosuppressive medications, she had FSGS on kidney biopsy, and experienced progressive decline in kidney function, reaching ESKD at age 7 years. She received a living related donor transplant and has never had recurrence of her disease.*

Question 2: What is the approximate likelihood that this child has a genetic form of NS?

a) 0%
b) 10%
c) 30%
d) 50%
e) 80%

For the answer to the question, see the following text.

Making a diagnosis of a Mendelian form of NS is important because it can affect clinical care, inform future family planning, and end diagnostic odysseys. However, what are the chances that a child with NS has a Mendelian cause? This is important information that helps guide the clinician's and parents' decision to order the relevant tests and/or calibrate their expectations of a positive result. Now we can more accurately estimate the prevalence of monogenic disease in pediatric NS because thousands of children around the world have been sequenced for many, if not all, of the most commonly implicated genes.

What are the chances that a child has a Mendelian form of NS? Children with congenital NS or SRNS are the patients who most likely have Mendelian forms of NS. Among these children, those with other affected family members are most likely to have a monogenic cause. Prevalence rates of 25% to 30% have been reported in pediatric cohorts in Australia, Europe, and the United Kingdom. However, these cohorts included children with congenital NS and those from endogamous unions. A prevalence of 6% to 11% has been observed in children in North America and Colombia, areas where there is more genetic admixture. The prevalence of monogenic NS decreases from birth to the later teen years. It then increases when autosomal dominant forms of NS begin to appear more frequently. The differing prevalence estimates by country are mostly thought to be a proxy for consanguinity or inclusion of children with congenital NS in the study.

By sequencing groups of children underrepresented in the existing literature, recent studies by our groups have provided additional insights on Mendelian NS in children. In 95 Brazilian children who had progressed to ESKD due to NS, we sequenced 24 NS genes. In predicting the known monogenic prevalence, on the one hand, we would expect a higher prevalence of Mendelian disease because we consider progression to ESKD to be an "extreme" phenotype of NS. On the other hand, given that the Brazilian population is highly admixed, particularly between those of sub-Saharan African, European, and indigenous ancestry, we would predict a lower prevalence of NS. We identified ~8% of this cohort with monogenic NS. This result may be explained in a couple of ways. A less likely explanation is that there are a group of novel Mendelian NS genes found in a substantial number of these patients that were not tested. It is more likely that there may be a burden of non-Mendelian genetic variation that alone or in combination with environmental variables contributes to NS in these populations.

In North America, we sequenced 20 known Mendelian NS genes in 311 affected patients enrolled in the Nephrotic Syndrome Study Network (NEPTUNE) cohort, of whom 95 were children. NEPTUNE enrolled patients undergoing a clinically indicated biopsy for the first time regardless of their response to immunosuppression. Of the 95 children, 6.3% had a putative known Mendelian form of NS. For the 36 children who did not achieve complete remission, the prevalence was 5.6%. There was no difference in achievement of complete remission between children with or without a putative Mendelian form of NS. According to genetic studies in pediatric SRNS in admixture populations in the United States and Brazil, as described, the likelihood

of the patient described in case 2 having a genetic form of SRNS is ~10%. The correct answer is therefore (b).

As we increasingly incorporate genomic information into our clinical practice, it will be critical to better understand the relationship between Mendelian NS and achievement of complete remission in individual patients with specific variants.

Rare CNV and NS

Rare deletions and duplications of the genome that overlap genes, a frequent genetic cause of congenital anomalies of the kidney and urinary tract (CAKUT), may also make a substantial contribution to pediatric NS. A CNV study of 419 children enrolled in the Chronic Kidney Disease in Children (CKiD) Study identified 31 children (7.4%) with pathogenic CNVs, of whom 5 had a clinical/histologic diagnosis of FSGS. Another study found pathogenic CNVs affecting known Mendelian NS genes, such as exon 23 to 29 deletion in *NPHS1* and exon 2 deletion in *NPHS2*.

Additional Readings

- Bierzynska A, McCarthy HJ, Soderquest K, et al. Genomic and clinical profiling of a national nephrotic syndrome cohort advocates a precision medicine approach to disease management. *Kidney Int.* 2017;91(4):937-947. ★ ESSENTIAL READING
- Feltran LS, Varela P, Silva ED, et al. Targeted next-generation sequencing in Brazilian children with nephrotic syndrome submitted to renal transplant. *Transplantation.* 2017;101(12):2905-2912.
- Mallett AJ, McCarthy HJ, Ho G, et al. Massively parallel sequencing and targeted exomes in familial kidney disease can diagnose underlying genetic disorders. *Kidney Int.* 2017;92(6):1493-1506.
- 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(6):1279-1289.
- Sampson MG, Gillies CE, Robertson CC, et al. Using population genetics to interrogate the monogenic nephrotic syndrome diagnosis in a case cohort. *J Am Soc Nephrol.* 2016;27(7):1970-1983.
- Trautmann A, Bodria M, Ozaltin F, et al. Spectrum of steroid-resistant and congenital nephrotic syndrome in children: the PodoNet registry cohort. *Clin J Am Soc Nephrol.* 2015;10(4):592-600.
- Verbitsky M, Sanna-Cherchi S, Fasel DA, et al. Genomic imbalances in pediatric patients with chronic kidney disease. *J Clin Invest.* 2015;125(5):2171-2178. ★ ESSENTIAL READING
- Wang F, Zhang Y, Mao J, et al. Spectrum of mutations in Chinese children with steroid-resistant nephrotic syndrome. *Pediatr Nephrol.* 2017;32(7):1181-1192.

Genetic Reclassification

Case 3. *You are asked to see a 9-year old white girl for a second opinion for management of her SRNS. She had proteinuria (4+) and microscopic hematuria on a urinalysis performed 8 months after she was noted to have edema. The NS did not respond to steroids and biopsy showed FSGS on light microscopy, normal immunofluorescence, and electron microscopy only significant for foot-process effacement. She did not achieve remission with tacrolimus or rituximab. There is no family history of kidney disease. She has normal hearing. You perform whole-exome sequencing using a clinical testing service. She has a heterozygous variant in collagen 4A3 (COL4A3) that is absent in the population and has been reported as causal for Alport syndrome.*

Question 3: What is her diagnosis?

a) Alport syndrome
b) Primary FSGS
c) Thin basement membrane disease
d) Collagen IVA glomerulopathy

For the answer to the question, see the following text.

With broad sequencing now increasingly available as a clinical tool, increasing numbers of genes are being sequenced in increasing numbers of patients. This raises risks for false positives and discovery of incidental noncausal variations. However, when a known gene associated with a given phenotype is found, there is substantial clinical benefit. In addition, large-scale sequencing is allowing us to make unexpected clinically meaningful discoveries. We briefly discuss the concepts of reclassification of a clinical diagnosis based on genetic results and phenotypic expansion of causal variants.

A child with NS with an FSGS pattern on biopsy may undergo targeted sequencing of known NS genes. If this test is negative, a treatment course of steroids and/or second-line immunosuppressive medications may continue for months or years with the rationale that absent a Mendelian diagnosis, there may be immune dysregulation that can be addressed with these medications. However, with expanded sequencing, we are now realizing that a number of children with FSGS may have Mendelian variants in genes originally discovered as causing kidney diseases other than SRNS/FSGS. We can refer to these genes as "phenocopying" the FSGS/NS.

In our clinic, among genes in which mutations phenocopy FSGS (Table 2), we have seen children with SRNS/FSGS diagnosed who were ultimately found to have pathogenic variants in *CLCN5*, the gene underlying Dent disease, and in *UMOD*, the gene encoding uromodulin and underlying familial juvenile hyperuricemic nephropathy and medullary cystic kidney disease. Genetically reclassifying these patients with FSGS lesions was clinically meaningful because we immediately stopped immunosuppressive medications.

Less anecdotally, a recent whole-exome sequencing study of 300 families with a clinical diagnosis of SRNS discovered a causal variant in 85 families. Among them, 11 (13%) were in a non-SRNS gene, including collagen type IV alpha genes, *CLCN5*, and genes implicated in cystinosis and Jeune syndrome. This molecular reclassification resulted in changes in management, screening for associated problems, and alterations in family counseling.

Table 2. Genes That Can Phenocopy SRNS/FSGS

Gene	Inheritance	Specific Disease
EYA1	AD	Branchio-oto-renal syndrome
COL4A3	AR	Alport syndrome
COL4A4	AR	Alport syndrome
COL4A5	X-linked	Alport syndrome
AGXT	AR	Hyperoxaluria
CLCN5	X-linked	Dent disease
CTNS	AR	Cystinosis
FN1	AD	Glomerulopathy with fibronectin deposits
GLA	X-linked	Fabry disease
WDR19	AR	Senior-Loken syndrome
PAX2	AD	Renal-coloboma syndrome
UMOD	AD	Medullary cystic kidney disease

Note: The list is of genes associated with other monogenic kidney diseases, but in which causal variants have been found in patients diagnosed with nephrotic syndrome.
Abbreviations: AD, autosomal dominant; AR, autosomal recessive; FSGS, focal segmental glomerulosclerosis; SRNS, steroid-responsive nephrotic syndrome.

Of note, there has been a phenotypic expansion of the clinical/histologic consequences of collagen IV causal variants, with multiple reports implicating them in FSGS without obvious abnormalities in the glomerular basement membrane. It may be ultimately appreciated that Alport syndrome is but one form of collagen IV–related glomerulopathy, which is the diagnosis of the patient described in case 3. She has SRNS with hematuria and has characteristics of FSGS on kidney biopsy, with no evidence of basement membrane disease abnormalities on electron microscopy. Heterozygous *COL4A3* variants have been implicated in collagen IVA glomerulopathy and as a phenocopy of FSGS, as in this case (meaning the answer is d).

Additional Readings

- Malone AF, Phelan PJ, Hall G, et al. Rare hereditary COL4A3/COL4A4 variants may be mistaken for familial focal segmental glomerulosclerosis. *Kidney Int.* 2014;86(6):1253-1259.
- Voskarides K, Damianou L, Neocleous V, et al. COL4A3/COL4A4 mutations producing focal segmental glomerulosclerosis and renal failure in thin basement membrane nephropathy. *J Am Soc Nephrol.* 2007;18(11):3004-3016.
- Warejko JK, Tan W, Daga A, et al. Whole exome sequencing of patients with steroid-resistant nephrotic syndrome. *Clin J Am Soc Nephrol.* 2018;13(1):53-62. ★ **ESSENTIAL READING**

Common Risk Variants in NS

Overview

Case 4. *An 8-year-old black girl is referred to your clinic by her pediatrician for evaluation of a 1-week history of periorbital and lower-extremity swelling. Her medical history is notable only for being born at week 30 of gestation for unknown reasons. Her family history is notable for a paternal uncle who had kidney disease of unknown cause diagnosed at age 24 years that progressed to ESKD at 28 years. The child is in the 50th percentile for height and weight and is normotensive. Laboratory measurements are remarkable for proteinuria (4+) and trace hematuria on urine dipstick, serum albumin level of 2.0 g/dL, total cholesterol level of 400 mg/dL, and estimated glomerular filtration rate (eGFR) of 60 mL/min/1.73 m^2.*

Question 4: What is the most likely genetic variant associated with her NS?

a) Causal variant in a gene previously implicated in Mendelian forms of NS
b) Causal variant in a novel Mendelian NS gene
c) Common risk variants in apolipoprotein L1 gene (*APOL1*)
d) Common risk variants in the HLA region

For the answer to the question, see the following text.

There is a spectrum of alleles beyond those that are rare and exonic. We now review the properties of common genetic variants associated with disease, their discovery in NS, and the subsequent characterization of their clinical and biological consequences.

As compared with rare, or novel, causal variants, common NS-associated genomic variants are present in healthy members of the population. They are most robustly discovered through a genome-wide association study (GWAS). In a typical GWAS, we use a "single-nucleotide polymorphism (SNP) chip" to genotype hundreds of thousands to millions of SNVs across the genomes of cases and controls of the same ethnicity (as a proxy of genetic ancestry). Sporadically affected individuals can be used for GWAS, and there is no need to include patients with familial disease or their parents. We then use simple statistics to identify SNVs that significantly differ in frequency between the 2 groups. SNVs that reach this threshold are colloquially referred to as risk alleles. We express their magnitude of effect on risk for disease as an odds ratio (OR). Because of linkage disequilibrium between SNVs, we observe a "risk locus," or a group of SNVs that are all significantly associated with disease.

Discovering the biological relevance of risk alleles discovered using GWAS is challenging for 2 major reasons. First, most GWAS risk alleles are noncoding and likely affect disease through changes in gene regulation. Second, because SNVs included on the genotyping chips for GWAS are chosen primarily to cover the genome and not based on function, it is most likely that the discovered risk allele identified is not the causal one. Together, this means that when a risk allele is discovered, further analyses must be done to discover: (1) the gene for which it regulates expression, (2) tissues and cell types in which it exerts its effects, and (3) the causal SNV at the locus.

However, even if the causal SNV, target gene, and/or tissue and its function is unknown, risk alleles can still serve as effective indicators of clinical outcomes. To identify the clinical effect of common risk alleles, we can first stratify patients with NS by the presence of this risk allele using additive (0 vs 1 vs 2) or recessive (0 and 1 vs 2) models. We can then seek to discover significant

association of risk alleles with clinical or molecular phenotypes.

NS in Black Children

There are racial disparities in NS and particularly FSGS, with black individuals more likely to have this condition. In terms of pediatrics, black children with FSGS are more likely to progress to ESRD and to do so more rapidly. Because of this increased risk for FSGS in African American individuals (who have varying proportions of African ancestry), investigators hypothesized that genetic variants associated with it lie in a region of the genome that is of greater African origin. This hypothesis was borne out, with the discovery that the excess FSGS risk in blacks was driven by 3 common exonic alleles in *APOL1* that arose about 8,000 years ago in Africa. These became more common through positive selection because in the heterozygous state, they are protective against trypanosome infections.

Two alleles are missense variants virtually always inherited together, collectively referred to as G1. The third allele is a 6–base pair in-frame deletion referred to as G2. In black Americans, the allele frequency of G1 is ~22%, and G2 is ~13%. The risk for FSGS from these *APOL1* variants follows a recessive model, with 2 risk alleles (termed a high-risk genotype) conferring the increased risk for disease. Altogether, black Americans with a high-risk genotype have a 12- to 15-fold increased odds of FSGS. In the pediatric nephrology clinic, we have found that 60% to 70% of African American children with NS harbor an *APOL1* high-risk genotype.

Given its importance, we compared baseline and longitudinal clinical characteristics between black children harboring a high-risk *APOL1* genotype versus a low-risk genotype (0 or 1 risk alleles) in the NEPTUNE and CKiD studies. Children with a high-risk genotype had a significantly: (1) older age of disease onset (CKiD: 11.5 vs 4.5 years and NEPTUNE: 14 vs 11 years), (2) lower eGFR at presentation (CKiD: 16 mL/min/1.73 m^2 less and NEPTUNE: 10 mL/min/1.73 m^2 less), and (3) increased inflammation on kidney biopsy. The high-risk genotype was also significantly associated with 3.2 times decreased odds of achieving complete remission of proteinuria and more rapid decline in eGFR. From a transplantation perspective, a number of other studies have found that a high-risk *APOL1* genotype of the donor, and not the recipient, is associated with worse kidney outcomes.

Its high prevalence also suggests that many children would benefit from treatments targeting the underlying mechanisms driving *APOL1*-associated NS. No such treatments exist yet, but work toward a mechanistic understanding is occurring through epigenetic, genomic, and transcriptomics studies in humans, human cell lines, and transgenic flies, fish, and mouse model systems. A proposed mechanism of the defective protein's pathogenicity includes serving as a cation channel, anion channel, or both in various cell membranes (eg, mitochondria and lysosome), causing fluxes of electrolytes leading to cell swelling and death. Other studies implicate the role of *APOL1* in abnormalities of protein trafficking in cells, endosomal functioning, and endosome-lysosome fusion in podocytes. It will ultimately be interesting to discover whether the negative effects of high-risk *APOL1* on the glomerular filtration barrier are through the same mechanism that makes it an effective weapon against trypanosomal infection.

Although a high-risk *APOL1* genotype confers high odds of developing FSGS and is present in 13% of African Americans, each person harboring it has only an estimated 4.3% lifetime risk for developing FSGS. This is an example of incomplete penetrance. It suggests that for people with a high-risk genotype to develop NS, they either need a second hit or removal of a protective factor. These factors can either be genetic or nongenetic. The clearest second hit is human immunodeficiency virus (HIV) infection, with at least a 30-fold increased odds of HIV-associated nephropathy in those with a high-risk genotype. In a case series of 11 patients who developed collapsing glomerulopathy after exogenous interferon administration, 100% of those genotyped (7/7) had the high-risk genotype. This fits the known biology of *APOL1*, for which expression is upregulated by interferons. There is significantly increased odds of prematurity in black children with glomerular disease and the high-risk versus the low-risk genotype, but the *APOL1* high-risk genotype was not associated with prematurity. More work with a preterm cohort needs to be done to determine whether a high-risk genotype and prematurity together result in increased odds of NS.

The patient in case 4 is a black girl who was born prematurely, has a family history of ESRD, and at the onset of NS symptoms presents with reduced eGFR. These characteristics make it more likely that she has *APOL1*-associated NS; thus, the answer is (c).

Identification of genetic or environmental modifiers of the *APOL1* high-risk genotype could be incredibly important. If there are modifiable modifiers, avoidance or augmentation of them could potentially prevent NS from developing. Even if they are nonmodifiable modifiers, if they can be measured, they could serve as markers to classify a child with a high-risk *APOL1* genotype at particularly elevated risk for developing NS who may then be under closer surveillance for the onset of glomerular damage.

Black children with NS are not routinely screened for the *APOL1* high-risk genotype. However, this would surely change in patients with NS if *APOL1*-targeted therapies are developed or modifiable factors are discovered that affect the progression of kidney disease in these children. There is currently no rationale for routine community screening of healthy black children for this risk genotype. However, if we ultimately discover modifiable or nonmodifiable factors that could inform us of which of the 13% of all black children with a high-risk genotype will develop kidney disease, community-based screening of *APOL1* in this population could be useful, with a follow-up intervention or increased monitoring as appropriate.

Additional Readings

- Divers J, Nunez M, High KP, et al. JC polyoma virus interacts with APOL1 in African Americans with nondiabetic nephropathy. *Kidney Int.* 2013;84(6):1207-1213.
- Freedman BI, Pastan SO, Israni AK, et al. APOL1 genotype and kidney transplantation outcomes from deceased African American donors. *Transplantation.* 2016;100(1):194-202.
- Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science.* 2010;329(5993):841-845. ★ ESSENTIAL READING
- Kopp JB, Smith MW, Nelson GW, et al. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. *Nat Genet.* 2008;40(10):1175-1184.
- Ng DK, Robertson CC, Woroniecki RP, et al. APOL1-associated glomerular disease among African-American children: a collaboration of the Chronic Kidney Disease in Children (CKiD) and Nephrotic Syndrome Study Network (NEPTUNE) cohorts. *Nephrol Dial Transplant.* 2017;32(6):983-990. ★ ESSENTIAL READING
- Nichols B, Jog P, Lee JH, et al. Innate immunity pathways regulate the nephropathy gene apolipoprotein L1. *Kidney Int.* 2015;87(2):332-342.
- Sampson MG, Robertson CC, Martini S, et al. Integrative genomics identifies novel associations with APOL1 risk genotypes in black NEPTUNE subjects. *J Am Soc Nephrol.* 2016;27(3):814-823.
- Tzur S, Rosset S, Shemer R, et al. Missense mutations in the APOL1 gene are highly associated with end stage kidney disease risk previously attributed to the MYH9 gene. *Hum Genet.* 2010;128(3):345-350.

Pediatric SSNS

As compared to SRNS, family-based studies have not identified monogenic causes of SSNS. Thus, it has been thought that the genetic architecture of SSNS is more likely to be polygenic/complex rather than Mendelian in nature. Successful GWAS in adults with 2 other glomerular diseases, membranous nephropathy and immunoglobulin A nephropathy, ultimately prompted similar approaches in SSNS.

Gbadegesin et al studied about 26,000 exonic SNPs with an allele frequency greater than 0.05 in 214 Sri Lankan children with NS (median age, 3 years) and 149 ancestry-matched controls. Although the sample size was small, the case phenotype was specific: responsive to oral steroid treatment within 8 to 12 weeks and classified as SSNS by their physicians. They discovered a significantly increased risk for SSNS with missense variants in *HLA-DQA1* and *HLA-DQB1*, which were in complete linkage disequilibrium. They estimated that these alleles explain only ~4.5% of the risk for SSNS, implying that other independent alleles remain to be discovered.

Building on this work, 2 GWAS in 2018 of SSNS, 1 from Japan and 1 from Europe and the United States, furthered our understanding of the genetic architecture of this condition. These studies both used genome-wide SNP arrays followed by imputation, which increased the number of genomic regions assessed for association with SSNS. The Japanese GWAS comprised 224 cases and 419 controls. It identified a significant locus in the *HLA-DR/DQ* region, with the lead risk allele conferring 2.8 times increased odds of NS. There were no other significant SNPs outside the HLA region or within it after conditional analysis. The lead SNP explained 9.7% of disease variance in Japanese children with SSNS.

The European-based transethnic meta-analysis comprised children of 3 different continental ancestries; European (144 cases), sub-Saharan African (56 cases), and North African (85 cases). Three independent regions associated with SSNS; all were in the HLA region, in the 3′ untranslated region of the transcript encoding *HLA-DQB1* (OR, 3.3), upstream of the start of the *HLA-DRB1* gene (OR, 2.2), and in the 3′ untranslated region of the transcript encoding *BTNL2* (OR, 3.5). The impact of these alleles among pediatric patients with NS in NEPTUNE was then assessed. The risk alleles were associated with decreased glomerular expression of *HLA-DRB5*, *HLA-DRB1*, and *HLA-DQB1*, younger age of onset (a phenomenon also observed in the European GWAS cohort), and increased odds of achieving complete remission, independent of histologic diagnosis.

Altogether, these population-based genome-wide studies of pediatric SSNS across worldwide cohorts demonstrate the key role of variation in the HLA region in its pathogenesis. They also place the genetic architecture of SSNS firmly within the realm of other diseases of immune dysregulation, such as diabetes mellitus. Future work will include continued fine mapping of the HLA antigen region and experimental work based on the discoveries already made. Continued GWAS of pediatric NS in independent cohorts with subsequent meta-analyses hold hope in identifying additional loci outside of HLA antigens contributing to the risk for SSNS in children.

Additional Readings

- Debiec H, Dossier C, Letouze E, et al. Transethnic, genome-wide analysis reveals immune-related risk alleles and phenotypic correlates in pediatric steroid-sensitive nephrotic syndrome. *J Am Soc Nephrol.* 2018;29(7):2000-2013.
- Gbadegesin RA, Adeyemo A, Webb NJ, et al. HLA-DQA1 and PLCG2 are candidate risk loci for childhood-onset steroid-sensitive nephrotic syndrome. *J Am Soc Nephrol.* 2015;26(7):1701-1710.
- Jia X, Horinouchi T, Hitomi Y, et al. Strong association of the HLA-DR/DQ locus with childhood steroid-sensitive nephrotic syndrome in the Japanese population. *J Am Soc Nephrol.* 2018;29(8):2189-2199.

Conclusion

It is an exciting time to consider childhood-onset NS from a genomic perspective. Mendelian- and polygenic-based strategies in familial and sporadic NS cases have led to the discovery of genes and genetic regions associated with disease. Technologic advances and the recruitment of thousands of children with NS worldwide have allowed us to conduct research to estimate its prevalence and discover clinical correlates. Also, there are now a number of testing

Box 3. Rationale for Recommending Genetic Testing in Children With NS

- Providing a precise diagnosis
  - ◇ Ending uncertainties
  - ◇ Expanding NS phenotypes
  - ◇ Reclassifying incorrectly diagnosed patients (eg, FSGS exists as part of phenotypic spectrum of Alport syndrome)
- Tailoring therapies
  - ◇ Reducing the duration, intensity, and collateral effects of drugs (steroids and immunosuppressants)
  - ◇ Potential may exist at some point to reveal targeted therapeutic options
- Predicting outcomes (as a consequence of genotypic-phenotypic correlation)
- Rationalizing decisions around transplantation
  - ◇ Choosing donors
  - ◇ Managing expectation of recurrence of NS
- Counseling family members
  - ◇ Making diagnoses of family members
  - ◇ Reproductive counseling
- Reduce need for kidney biopsy in some cases (particularly with family members of proband with NS who themselves develop proteinuria)

Abbreviations: FSGS, focal segmental glomerulosclerosis; NS, nephrotic syndrome.

strategies available to clinicians to sequence or genotype their patients to discover whether they have a known genetic form of their disease. Altogether, these advances have provided a strong rationale to perform genetic testing in at least some children with NS (Box 3), and we expect this proportion to increase over time.

From a clinical perspective, the volume of the genomic data generated and the complexity of its interpretation can be overwhelming for pediatric and adult nephrologists. Adding to this, genomic studies for NS are no longer solely ordered and interpreted by geneticists and genetic counselors. Thus, it is incumbent on members of our specialty to gain comfort with an "applied genomics" approach to NS as we seek to actualize the benefits of genomics discovery for our patients with childhood-onset NS. We hope that this review has been helpful toward achieving this goal.

Finally, as we continue to bring applied genomics to the NS clinic, there are innumerable opportunities to contribute to or drive efforts for further genomic discovery in pediatric NS. This includes discovering additional genes and loci associated with NS, discovering and/or refining clinical correlates for children harboring known NS genetic variants, and recruiting additional affected patients into research studies, which will increase our power for discovery. These activities will be of particularly high impact for nonwhite patients, who are currently underrepresented in genetic research, and those from countries outside of the United States and Europe, where genomic discovery in NS has not traditionally taken place. By coming together worldwide in these efforts, we should ultimately be able to improve the health of children with NS.

Supplementary Material

Supplementary File (PDF)

Table S1: A selection of websites that can help to understand the meaning of a given gene variant.

Table S2: Genes implicated in monogenic forms of steroid-resistant or partially treatment-sensitive NS, their mode of inheritance, related phenotype, and specific findings.

Table S3: Genetic testing modalities.

Article Information

Authors' Full Names and Academic Degrees: Andreia Watanabe, MD, Luciana S. Feltran, MD, and Matthew G. Sampson, MD, MSCE.

Authors' Affiliations: Department of Pediatrics, Pediatric Nephrology Unit, Instituto da Crianca, University of Sao Paulo School of Medicine (AW); Nephrology Department, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil (LSF); and Department of Pediatrics-Nephrology, 4 Center for Computational Medicine and Bioinformatics, University of Michigan School of Medicine, Ann Arbor, MI (MGS).

Address for Correspondence: Matthew G. Sampson, MD, MSCE, 3560B MSRB2, 1150 W Medical Center Dr, University of Michigan School of Medicine, Ann Arbor, MI 48109. E-mail: mgsamps@med.umich.edu

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