The etiology of congenital nephrotic syndrome: current status and challenges

Jing-Jing Wang, Jian-Hua Mao
Hangzhou, China

Background: **Congenital nephrotic syndrome (CNS), defined as heavy proteinuria, hypoalbuminemia, hyperlipidemia and edema presenting in the first 0-3 months of life, may be caused by congenital syphilis, toxoplasmosis, or congenital viral infections (such as cytomegalovirus). However, the majority of CNS cases are caused by monogenic defects of structural proteins that form the glomerular filtration barrier in the kidneys. Since 1998, an increasing number of genetic defects have been identified for their involvements in the pathogenesis of CNS, including *NPHS1*, *NPHS2*, *WT1*, *PLCE1*, and *LAMB2*.**

Data sources: **We searched databases such as PubMed, Elsevier and Wanfang with the following key words: congenital nephrotic syndrome, proteinuria, infants, neonate, congenital infection, mechanism and treatment; and we selected those publications written in English that we judged to be relevant to the topic of this review.**

Results: **Based on the data present in the literature, we reviewed the following topics: 1) Infection associated CNS including congenital syphilis, congenital toxoplasmosis, and congenital cytomegalovirus infection; 2) genetic CNS including mutation of *NPHS1* (Nephrin), *NPHS2* (Podocin), *WT1*, *LAMB2* (Laminin-β2), *PLCE1* (NPHS3); 3) Other forms of CNS including maternal systemic lupus erythematosus, mercury poisoning, renal vein thrombosis, neonatal alloimmunization against neutral endopeptidase.**

Conclusions: **At present, the main challenge in CNS is to identify the cause of disease for individual patients. To make a definitive diagnosis, with the exclusion of infection-related CNS and maternal-associated disorders, pathology, family history, inheritance mode, and other accompanying congenital malformations are sometimes, but not always, useful indicators for diagnosing genetic CNS. Next-generation sequencing would be a more effective method for diagnosing genetic CNS in some patients, however, there are still some challenges with next-generation sequencing that need to be resolved in the future.**

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Key words: **congenital infection; congenital nephrotic syndrome; mono-genetic mutation; next-generation sequencing**

Review article

Introduction

Primary nephrotic syndrome, described as the tetralogy of massive proteinuria, hypoalbuminemia, hyperlipidemia and edema, is the most common glomerular disorder in children, with an incidence of approximately 1-3 per 100 000 children <16 years of age.[1] Furthermore, congenital nephrotic syndrome (CNS), defined as nephrotic syndrome (NS) presenting in the first 3 months of life, is a rare glomerular disease worldwide, compared with infantile nephrotic syndrome (appears 4-12 months after birth). Nephrotic syndrome manifesting after 3 months of age is called childhood nephrotic syndrome. The rationale for this classification is based on the difference in etiology,[2] clinical features and treatment strategy between CNS and late-onset NS, though a great deal of published evidence has suggested that massive proteinuria by a particular gene defect or pathogen can manifest at various ages throughout the lifespan.[3]

The etiology of CNS is heterogeneous, and genetic defects account for the vast majority of CNS cases. Infections, such as congenital syphilis and toxoplasmosis, are possible pathogens, especially in developing countries. Infection should be excluded as the cause of CNS before the establishment of genetic CNS because it is important for selecting a treatment strategy and for long-term prognosis in these cases.

In our opinion, the current main challenge for CNS is how to identify the cause of CNS for the individual

Author Affiliations: Department of Nephrology, Children's Hospital of Zhejiang University School of Medicine, Hangzhou, China (Wang JJ, Mao JH)

Corresponding Author: Jian-Hua Mao, Department of Nephrology, Children's Hospital, Zhejiang University School of Medicine, Hangzhou 310006, China (Tel: 0086-571-87061007; Fax: 0086-571-87033296; Email: maojh88@126.com)

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patient. Establishing a treatment strategy is also a major challenge, but it is not the primary problem because it depends on the cause of CNS. For that reason, the objective of our study is to list the possible causes of CNS, to identify the causes before the start of therapy, and to exclude false causes for CNS, such as CMV infections in some patients, before the initiation of therapy. In the present review, we summarized the current knowledge of genetic and non-genetic causes of CNS, the rational scheme for molecular sequencing, and current problems and challenges.

Infection-associated CNS

Congenital syphilis

Congenital syphilis has long been known to cause CNS[4] and it is common in developing countries.[5] Early treatment with penicillin can be curative. Neonates with congenital syphilis may present with fever, hepatosplenomegaly, persistent rhinitis, neurosyphilis, hepatitis, anemia, erythematous patches with superficial bullae, or desquamations. The extent of clinical renal involvement may vary from microscopic hematuria to more significant features, such as nephrotic syndrome.[6] Nephrotic syndrome generally appears between the 2 and 3 months of age.[7] Niemsiri et al[4] reported a 2.4% prevalence of congenital syphilis that caused CNS among 455 neonate or infants in Thailand. Membranous nephropathy is a common finding in renal biopsy, with immune deposits in the region of the glomerular basement membrane; this observation implicates the immune system in the pathogenesis of syphilis-associated CNS. If the diagnosis was made early, the prognosis is good because antimicrobial therapy, usually penicillin, is curative, provided that irreversible renal lesions have not developed.[7]

Congenital toxoplasmosis

Toxoplasmosis is a rare cause of nephrotic syndrome, with the majority of cases being associated with congenital infection.[8,9] Roussel et al[10] reported a case of a 1-month-old infant presenting with congenital toxoplasmosis associated with nephrotic syndrome and microscopic hematuria. Percutaneous renal biopsy showed a diffuse mild increase in mesangial cells and matrix, but immunofluorescence was negative. Fan et al[11] reported that a 3-month-old girl presented with massive proteinuria, anasarca, hypoalbuminemia and positive serum antibody IgM for toxoplasmosis. Her mother also presented with positive serum IgM antibody to toxoplasmosis. After treatment with spiramycin for 3 weeks, the patient became negative for proteinuria, and serum toxoplasmosis IgM antibody titer will vanish simultaneously. Beale et al[12] described two infants who had extensive glomerulosclerosis manifested by nephrotic syndrome, severe oliguria, and progressive renal failure. Both patients had intrauterine infections. Therefore, screening for congenital toxoplasmosis infection is necessary for hing-risk CNS patients.

Congenital cytomegalovirus infection

An association of neonatal cytomegalovirus (CMV) infection and CNS has also been reported.[3,13-15] In many cases, diffuse mesangial sclerosis (DMS) in histology is often found and the patients clearly respond to ganciclovir therapy. Besbas et al[3] described CNS associated with cytomegalovirus infection in a 2-month-old girl. Histological examination on renal biopsy showed diffuse mesangial sclerosis and cytomegalic inclusion bodies in the tubular cells and in some glomeruli. Cytomegalovirus (CMV) polymerase chain reaction (PCR) titer in the serum was high. Remission of pulmonary and renal symptoms was achieved with ganciclovir in 3 weeks. No recurrence of proteinuria was observed during the 14-month follow-up period. Their findings suggested a causal relationship between congenital nephrotic syndrome and CMV infection.

On the contrary, Frishberg et al[13] reported a girl with CNS associated with CMV infection; and histological findings on renal biopsy suggested a causal relationship between CNS and CMV infections. However, she was subsequently found to be homozygous for a nonsense mutation in the *NPHS2* gene encoding podocin (R138X), which is the true cause of her CNS. So when antiviral treatment is not effective in the patient with cytomegalovirus infection and CNS, one should consider other causes of CNS, especially the genetic factors.

Other pathogens

About 40% of all HIV-infected children in the United States present with renal complications.[13] Massive proteinuria and nephrotic syndrome usually appear in children older than 1 year, but no affected infants have been reported.[15,16] Severe proteinuria is more prevalent in African children.[17] To date, no CNS cases caused by HIV infection have been reported, but this remains a diagnostic consideration in newborn and young infants.

According to some literature,[18,19] Hepatitis B virus-related nephropathy can occur at any age but is more common in children. Furthermore, rare infants with CNS associated with congenital rubella have been described.[20,21]

Current problems for infection-related CNS

Using the present diagnostic procedure, the incidence of infection-related CNS may be over-estimated.

congenital syphilis,[6] toxoplasmosis,[9,10] HIV[22] and cytomegalovirus[23,24] infection have long been known to cause congenital nephrotic syndrome. Although other pathogens, such as rubella, hepatitis B virus, and hemoplasmodium, are reported to possibly cause congenital nephrotic syndrome in some literatures,[2] their roles in the pathogenesis of CNS remained to be identified further. At present, no causal relationship between these pathogens and CNS has been established.

As the kidney is the major organ of the excretory system, it is reasonable that many pathogens can be detected in the renal tubular lumen, tubular epithelial cell body, and especially in the glomerular capillary loops. The neonatal and early infant period is such a critical time when many pathogens may enter into the physical body of a host. Some of these pathogens may cause the given disease in the host body but some may not, because some pathogens enter and exit via the kidney and other excretory organs. For that reason, the diagnosis of a pathogen-related glomerulopathy may not be established even if the patients presented with signs of glomerulopathy and evidence of the deposited pathogen is simultaneously detected in the kidney. Therefore, response to treatment would be the important criterion for confirming the diagnosis of a pathogen-related glomerulopathy.

Frishberg et al[13] provided a good example in 2003. They described presumed CMV-associated CNS in a girl based on the histological findings on renal biopsy with a single inclusion body in the distal tubule. The nephrotic syndrome was not improved after treatment with intravenous ganciclovir, and her renal function deteriorated, reaching end-stage renal disease at the age of 21 months. She was finally identified to have a homozygous R138X mutation in the *NPHS2* gene. The conclusion from this report reinforces the idea that one should be cautious when making the diagnosis of infection-related CNS, especially when pathogen-specific treatment is not effective in the patient.

Other forms of non-genetic CNS

In addition to infections, non-genetic CNS has been associated with maternal systemic lupus erythematosus, mercury poisoning,[25] renal vein thrombosis,[26,27] and more recently with neonatal alloimmunization against neutral endopeptidase present on podocytes.[28]

Debiec et al[29] reported that anti–neutral endopeptidase (NEP) antibodies produced by a pregnant woman were transferred to her fetus, in which a severe form of membranous glomerulonephritis developed prenatally. The mother had a deficiency of NEP. Further, they identified that, during pregnancy, the absence of the NEP protein induces an alloimmunization process against NEP presented by fetal cells.[30] The fetal podocyte insult and ensuing nephron loss could lead to chronic renal failure in early adulthood. Alloimmunization against NEP should be considered as a leading cause of membranous glomerulopathy early in life. This new disease might also account for idiopathic chronic renal failure detected during adolescence in individuals who can be identified by searching for anti-NEP antibodies in their mother and by metallomembrane endopeptidase gene mutation analysis.

Genetic forms of CNS

Since 1998, more and more genetic defects have emerged

Table 1. The principle genes involved in congenital nephrotic syndrome and in associated syndromes

Genes	Locus	Protein	Phenotype
AD			
WT1	11P13	Wilms tumor 1	IDMS, DDS, Frasier syndrome, WAGR syndrome, ISRNS
LMX1B	17q11	Lim homeobox transcription factor 1-β	Nail-patella syndrome
INF2	14q32.33	Inverted formin-2	FSGS
CD2AP	6p12	CD2-associated protein	FSGS (adult)
AR			
NPHS1	19q13.1	Nephrin	CNF
NPHS2	1q25-31	Podocin	Idopathic CNS, SRNS
LAMB2	3p21	Laminin β2 chain	Pierson's syndrome
PLCE1	10q23	Phospholipse C epsilon 1	SRNS, DMS
PDSS2	6q21	Decaprenyl disphosphate synthase, subunit 2	NS with Leigh syndrome
ITGA3	17q21.33	Integrin α3	NS with intestitial lung disease
ARHGDIA	17q25.3	Rho GDP dissociation in inhibitor 2	Idiopathic CNS
SCARB2	4q21.1	Scuvenger receptor class B, number 2	Action myoclonus-renal failure syndrome
Unknown			Galloway-Mowat syndrome

AD: autosomal dominant; AR: autosomal recessive; IDMS: idiopathic diffuse mesangial sclerosis; DDS: Denys-Drash syndrome; WAGR: Wilm's tumor, aniridia, genitourinary abnormalities and mental retardation; FSGS: focal segmental glomerulosclerosis; CNS: congenital nephrotic syndrome; CNF: CNS of the Finnish type; SRNS: steroid-resistant nephrotic syndrome; DMS: Denys-Drash syndrome.

for the pathogenesis of CNS,[31] and these genetic defects account for the great majority of CNS.[28,32-35] According to the inheritance model, they may be divided as autosomal recessive (AR) or autosomal dominant (AD) model (Table 1).

NPHS1

CNS of the Finnish type (CNF; MIM No. 256300) is the most common cause of CNS and is named because of its high incidence in Finland of 1:8200 live births.[36] And it is the a recessively inherited disorder firstly described in highly inbred Finnish communities.[36] By positional cloning,[31] CNF was shown to be caused by mutations in *NPHS1*. CNF is characterized by massive proteinuria at birth, a large placenta, marked edema, premature birth and mutations in *NPHS1* present with NS in the first week of life.[33] Irregular microcystic dilatation of the proximal tubules is the most typical feature in histology. Mutations in the *NPHS1* gene encoding nephrin,[31] one of the most important molecules in maintaining the structural and functional integrity of the slit diaphragm and glomerular filtration barrier, are the most common cause for CNS worldwide.

The Finmajor mutation (nt121delCT, L41fsX91) and Finminor mutation (c.3325 C>T, R1109X) in the *NPHS1* gene were the first mutations to be discovered and the most prevalent mutations of CNF in the Finnish population (98% of cases).[31] However, these mutations are also found in other ethnic groups.[28] Screening for *NPHS1* mutations in patients of non-Finnish origin has shown that the frequency of *NPHS1* mutations is lower than that in Finnish patients, accounting for 39%-50% of non-Finnish cases with CNS.[37] On the other hand, rare cases with a manifestation beyond the age of 90 days have also been published, indicating that different mutations in *NPHS1* might cause a spectrum of clinical severity.[38,39] To date, 173 different mutations in *NPHS1* have been described (http://www.biobase-international.com). The present data demonstrate that the spectrum of *NPHS1* mutations is still expanding, involving new exons, in patients from diverse ethnic backgrounds all over the world.

The course of the disease is progressive, leading to end-stage renal disease by 2-3 years of age. Kidney survival is worse in patients with *NPHS1* mutations compared with *NPHS2* gene mutations.[33] Maori children with congenital nephrotic syndrome in New Zealand exhibit a moderate clinical course compared with Caucasian children. Maori patients with a defined nephrin mutation had significantly longer patient and renal survival than Caucasians.[40] This result implied that the prognosis of CNF varied within different ethnic backgrounds. One recent study[41] found that the female patients with *NPHS1* mutations survived longer than their male counterparts, but that gender did not affect age at diagnosis, and an earlier study[42] reported that female CNS patients with *NPHS1* mutations had slightly longer renal survival than males. Meanwhile, *NPHS1* mutations can cause childhood-onset steroid-resistant nephrotic syndrome.[38]

NPHS2

Podocin is a member of the stomatin family and localizes to lipid rafts. Its mutation induces injury in part via its effects on nephrin and the actin cytoskeleton.[43] More than 100 pathogenic *NPHS2* mutations have been reported that involve nonsense and frameshift mutations in exons. Though, mutations in *NPHS2* are typically responsible for childhood-onset steroid-resistant nephrotic syndrome,[33] even in young adult patients, complete loss of function may significantly alter glomerular development and cause CNS.[41,42,44] Hinkes et al[45] investigated the genotype–phenotype correlation between podocin mutations and age of onset, and concluded that the onset of nephrotic syndrome in patients with truncating or homozygous R138Q mutations is significantly earlier than for any other podocin mutations. Consistent with the major role of nephrin at the slit diaphragm, *NPHS1* mutations are associated with an earlier onset of disease and worse renal outcomes than *NPHS2* mutations.[33] Sako et al[46] reported in 13 unrelated Japanese patients with CNS: 4 patients carried an *NPHS1* mutation, 2 carried an *NPHS2* mutation, and neither *WT1* or *ACTN4* mutations were found in the other patients. Maruyama et al[47] sequenced 11 Japanese children with steroid resistant FSGS, and no causative mutations were detected.

WT1

The Wilms' tumor suppressor gene (*WT1*), identified in 1990, encodes a transcription factor that regulates the expression of many genes through DNA binding. It plays a critical role during kidney and genital development and, when mutated, in the occurrence of kidney tumors and glomerular diseases.

Mutation of *WT1* could potentially result in the following: WAGR syndrome,[48] Denys-Drash syndrome (DDS),[49] the syndrome characterized by the association of early onset nephrotic syndrome progressing rapidly to end-stage renal disease (ESRD), male pseudohermaphroditism and Wilms' tumor, isolated diffuse mesangial sclerosis (IDMS),[50] the syndrome characterized by the glomerulopathy similar to the DDS nephropathy but without the other elements of the triad; and Frasier syndrome, characterized by its association with male pseudohermaphroditism, gonadoblastoma

and progressive glomerulopathy. Furthermore, *WT1* mutations may be responsible for the occurrence of a glomerulopathy, with isolated steroid resistant nephrotic syndrome (ISRNS) by focal segmental glomerular sclerosis (FSGS), in genetically female patients.[51] Among these complex associations between *WT1* mutation and clinical glomerulopathy, early onset of proteinuria and congenital nephrotic syndrome may be present in patients with IDMS, ISRNS and DDS. For that reason, in CNS patients with or without genitourinary malformations, it is necessary to screen for *WT1* gene mutation.

LAMB2

Laminins are a large family of conserved, multidomain trimeric basement membrane proteins that contribute to the structure of extracellular matrix and influence the behavior of associated cells.[52] *In vitro* protein and cell culture studies, gene manipulation in animals, and laminin gene mutations in human diseases have provided insight into the specific functions of some laminins. According to literature, Laminin-β2 chain is essential for postnatal GBM function.[53,54] Mutations in human *LAMB2* lead to Pierson syndrome, a severe and often early lethal disorders with central nervous system and eyes involvements. Pierson syndrome is a rare autosomal recessive disease characterized by congenital nephrotic syndrome/diffuse mesangial sclerosis, distinct ocular abnormalities including microcoria (small pupils), muscular hypotonia, and impairments of vision and neurodevelopment.[54] Children affected by Pierson syndrome usually die within days or weeks after birth from renal failure. *LAMB2*-null mice appear to have normal glomeruli and GBMs, but neonatally severe proteinuria develops which causes early lethality.[55] Missense mutations of *LAMB2* present with a spectrum of symptoms ranging from isolated early-onset NS to intermediate phenotypes,[56] whereas patients with truncating *LAMB2* mutations present with the full syndromic phenotype of Pierson syndrome with NS, diffuse mesangial sclerosis, distinct eye anomalies, and mental retardation.[55] Hinkes et al[37] reported that in 46 cases of CNS, mutations were found in *NPHS1*, *NPHS2*, *WT1*, or *LAMB2* genes in 84.8% (39 of 46) of all families. The distribution for *NPHS1*, *NPHS2*, *WT1*, and *LAMB2* was: 39.1% (18 of 46), 39.1% (18 of 46), 2.2% (1 of 46), and 4.4% (2 of 46) in Europe.

Zhao et al[53] reported the first Chinese case of Pierson syndrome, which presented with childhood-onset heavy proteinuria, bilateral miosis and nystagmus. Two novel mutations were identified, C757fsX767 and P1413fsX1451, which predicted truncated proteins of laminin-β2. Further, Togawa et al[57] described the first Japanese case of Pierson syndrome with congenital nephrotic syndrome and bilateral microcoria at birth, and the patient developed end-stage renal disease at 2 months of age. Direct sequencing for *LAMB2* revealed the compound heterozygous mutations c.3974_3975insA (p.N1325KfsX1331, maternal, novel) in exon 25 and c.4519C→T (p.Q1507X, paternal) in exon 27.

Galloway-Mowat syndrome (GMS) is a rare autosomal recessive disorder characterized by early-onset nephrotic syndrome and microcephaly with various anomalies of the central nervous system. GMS likely represents a heterogeneous group of disorders with hitherto unknown genetic etiology.[58] The clinical phenotype, to some extent, overlaps with that of Pierson syndrome.

PLCE1 (NPHS3)

Diffuse mesangial sclerosis (DMS) is characterized by mesangial expansion and sclerosis that evolves towards obliteration of the capillary lumen and contraction of the glomerular tuft. This type of renal histology has been described as part of syndromes such as Denyse Drash syndrome or Pierson syndrome, caused by mutations in the *WT1* and *LAMB2* genes, respectively. More recently, *PLCE1* mutations have been found as a novel cause of DMS.[59] In this study,[59] Hinkes referred to a 2-month-old infant presenting with steroid-resistant but Cyclosporine A-sensitive nephrotic syndrome, and proteinuria remained in remission at 13 years during follow-up. Another patient with 1-year onset of steroid sensitive nephrotic syndrome was identified to have a homozygous p.Q1854X mutation. Gilbert et al[60] described a phenotypically completely normal adult that carried a pathogenic homozygous mutation, while two of his children presented with congenital or infantile nephrotic syndrome and were homozygous for a four-base pair deletion in exon 3 of the *PLCE1 gene*, which created a premature translational stop codon. Their results stated that persons with these mutations in *PLCE1* may have the phenotype of a completely normal adult, possibly due to other genes or environmental factors modifying the effect of *PLCE1* mutations.[61] Therefore, mutation within *PLCE1* is not always sufficient to cause diffuse mesangial sclerosis.

PDSS and *COQ*

Only one case of infant-onset nephrotic syndrome with pathogenic mutations in *PDSS2* confirms the molecular and clinical heterogeneity of primary CoQ10 deficiency reported by Lopez et al.[62]

ITGA3

The laminin-binding integrin, $\alpha 3\beta 1$, is expressed at high levels in lung epithelium and in kidney podocytes. In

podocytes, $\alpha 3\beta 1$ associates with the tetraspanin, CD151, to maintain a functional filtration barrier. Nicolaou et al[63] reported on a patient homozygous for a novel missense mutation in the human *ITGA3* gene, which caused fatal interstitial lung disease and congenital nephrotic syndrome. Further, Shukrun et al[64] described a patient within the initial cohort harboring an *ITGA3* mutation resulting in a unique phenotype at birth, including severe unilateral renal hypodysplasia. Their studies support the role of integrin $\alpha 3\beta 1$ in maintaining the functional integrity of podocytes and the glomerular filtration barrier.

CD2AP

Very little is known about *CD2AP* in human kidney diseases, as yet, only one heterozygous *CD2AP* mutation detected in two adult patients with primary FSGS has been reported.[65] To date, no case of CNS reported with a *CD2AP* mutation.[66]

NEPH1

No published study reported on patients with proteinuria and only animal models reported and association between proteinuria and glomerulopathy.

The challenges for genetic CNS

During the past few years, our knowledge of the genetic and non-genetic basis of CNS has greatly increased, although there are still some problems to be elucidated in future.

As mentioned above, genetic forms of CNS are commonly caused by mutations of *NPHS1*, *NPHS2*, *WT1*, *LAMB2*, or *PLCE1*. In addition to these genes, there are some reports on combinations of CNS/early onset of proteinuria and extra-renal defects, including CNS-associated mitochondrial cytopathy,[67] nail-patella syndrome[68] (LMX1B), congenital disorder of glycosylation type I,[69] Herlitz junctional epidermolysis bullosa,[70] mutations in coenzyme Q10 biosynthesis monooxygenase 6 (COQ6) producing CNS with sensorineural deafness,[71] *ITGA3* mutation,[63] *ITGB4* mutation,[72] *MYH9/APOL1* mutation,[73] *SCARB2* mutation,[74] *MafB* mutation,[75] *CD151* mutation,[76] *PTPRO* mutation,[77] *SMARCAL1* mutation,[78] *ARHGDIA* mutation,[79,80] *ARHGAP24* mutation,[81] *INF2* mutation,[82] and others. The facts that CNS could be caused by mutations from many renal-specific or universally expressed genes make genetic screening more difficult and time-consuming. Table 2 summarizes the important published papers with columns of country, number of cases assessed and genetic defects found.

Pathology is not at all diagnostic for different forms of CNS. The value of kidney biopsies in distinguishing different genetic disease etiologies is limited. As summarized by Liapis,[85] minimal change diseases (MCD) can be found in CNS patients with *NPHS1*, *NPHS2* and *WT1* mutations, diffuse mesangial sclerosis (DMS) can be found in CNS patients with *WT1*, *LAMB2* and *PLCE1* mutations, FSGS be found in CNS patients with *NPHS2*, *CD2AP*, *WT1* and *PLCE1* mutations, while collapsing glomerulopathy can be found in CNS patients with *COQ2* mutations. For these reasons, pathological findings may overlap within different gene mutations in patients with CNS or early-onset of nephrotic syndrome.

Family history and inheritance mode

Genes causing familial FSGS may also participate in sporadic versions of FSGS.[85] Dominant mutations of *ACTN4* are associated with early-onset proteinuria, and dominant mutations of *WT1* are also associated with Denys-Drash syndrome (DDS), Frasier syndrome, WAGR syndrome and IDMS, while autosomal-recessive CNS might be caused by mutations in *NPHS1*, *NPHS2*, *LAMB2*, *PLCE1* and *COQ2*, to name a few. Thus, the family history and inheritance model is only occasionally useful for differentiation of patients with family history.

Extra-renal phenotypes do not always present within the first 3-months of life due to different penetration rates of disease-causing genes. For example,

Table 2. The important published papers on congenital nephrosis syndrome

Authors	Year of publication	Countries	No. of CNS (A/B/C)*	Genetic defects
Cil O[35]	2015	Turkey, Iran, etc	80/80/58	*NPHS1, NPHS2, WT1, LAMB2*
Trautmann A[34]	2015	Europe, the Middle East, and Latin America	98/83/55	*NPHS1, WT1, NPHS2, PLCE1, SMARCAL1, LAMB2, COQ6*, etc
Kari JA[83]	2014	UK	31/9/7	*NPHS1, PLCE1*
Santin S[84]	2011	Spain	15/15/15	*NPHS1, NPHS2, WT1*
Machuca E[33]	2010	Europe, Africa, etc	117/117/93	*NPHS1, NPHS2, WT1, LAMB2*
Hinkes BG[37]	2007	European Cohort	54/46/39	*NPHS1, NPHS2, WT1, LAMB2*
Sako M[46]	2005	Japan	13/13/6	*NPHS1, NPHS2*

*: A is the number of reported patients with congenital nephrotic syndrome, B is the number of patients screened, and C is the number of patients with causative mutations.

WT1 mutation causes congenital nephrotic syndrome with IDMS, ISRNS and DDS, which means that some CNS patients carrying *WT1* mutations may present with male pseudohermaphroditism and Wilms' tumor, while some present with isolated DMS or SRNS only. Another sample comes from the *LAMB2* gene mutation. Patients with mutations of *LAMB2* present with a spectrum of symptoms ranging from isolated early-onset NS to the full syndromic phenotype of Pierson syndrome with CNS, diffuse mesangial sclerosis, distinct eye anomalies, and mental retardation.[55] Therefore, different penetrating rates of genes alter the phenotype of affected patients, including the onset of disease.

Furthermore, the phenotype of monogenic CNS may be modified by the other underlying genes with compensatory functions. The presence of an additional heterozygous polymorphism in *WT1* in a patient with *NPHS1* mutation was associated with earlier-onset disease, supporting modification of phenotype through genetic epistasis.[86] Lowik[87] et al described a tri-allelic hit that was found in a patient carrying compound heterozygous *NPHS2* mutations and a heterozygous *NPHS1* mutation. We also reported on 5 children carrying both *NPHS1* and *NPHS2* mutations.[39]

Next-generation sequencing for molecular diagnosis

The traditional genetic test is based on the understanding that mutations in single gene defects have invariably been in the coding regions of the gene of interest, interpretation of negative results is more problematic, since the patient may have either a mutation in non-coding regions of candidate genes or mutations in novel genes. The rapid technological advances in "next generation sequencing (NGS)" will likely decrease the need for this candidate gene approach and may replace it as the test of choice in the near future. Through NGS, scientists are able to sequence hundreds of thousands of genes and, in some cases, entire genomes simultaneously to generate extensive data in a timely and cost-effective manner, compared with Sanger sequencing.[88] McCarthy et al[86] successfully conducted the next generation sequencing to screen 446 genes, including the 24 genes known to be associated with hereditary steroid-resistant nephrotic syndrome. Their study showed that NGS of pediatric steroid-resistant nephrotic syndrome patients is accurate and revealing. This analysis should be considered part of the routine genetic workup of diseases such as CNS or childhood-onset steroid-resistant nephrotic syndrome, where the chance of genetic mutation is high but requires sequencing of multiple genes. The figure shows a variant analysis pathway in patients with congenital nephrotic syndrome.

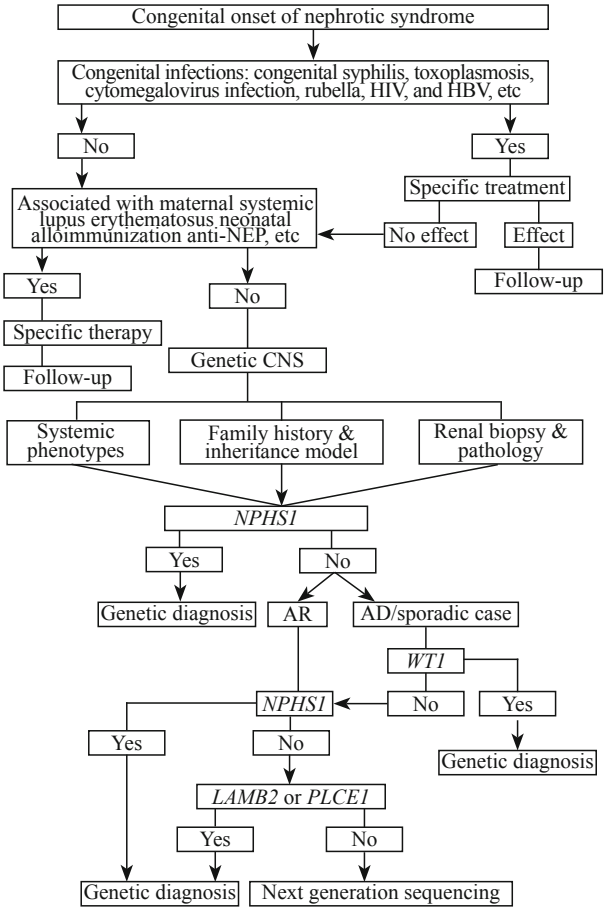

Fig. Flow diagram shows the variant analysis pathway in patients with congenital nephrotic syndrome. HIV: human immunodeficiency virus; HBV: hepatitis B virus; NEP: neutral endopeptidase; CNS: congenital nephrotic syndrome; AD: autosomal dominant; AR: autosomal recessive.

Processing the large amount of data generated from NGS can be costly and complicated, and data storage and security may raise logistical and ethical concerns.[89] Most importantly, it is still troublesome to identify whether a novel variant is phenotype-causing or not. Gilbert et al[60] described three children from one consanguineous kindred of Pakistani origin with diffuse mesangial sclerosis who presented with congenital or infantile nephrotic syndrome. All affected children were homozygous for a 4-base-pair deletion in exon 3 of the *PLCE1* gene, which created a premature translational stop codon. Analysis of the asymptomatic father of two of the children revealed that he was also homozygous for the same mutation. They concluded that this non-penetrance may be due to compensatory mutations at a second locus and that mutation within *PLCE1* is not always sufficient to cause diffuse mesangial sclerosis. Taken together, these reports suggest that identifying a variant as disease-causing is sometimes very difficult for clinicians.

Conclusions

Congenital nephrotic syndrome, defined as the nephrotic syndrome presenting in the first 0-3 months of life, may be caused by congenital syphilis, toxoplasmosis, viral infection, or more importantly, by genetic defects in structural proteins that form the glomerular filtration barrier. Since 1998, an increasing number of genetic defects have emerged in the pathogenesis of CNS, including *NPHS1*, *NPHS2*, *WT1*, *LAMB2* or *PLCE1*. To make a definitive diagnosis, next-generation sequencing would be a more effective method than renal pathology, family history and inheritance mode, and extra-renal phenotypes in patients with genetic CNS. However, there are challenges with next-generation sequencing that need to be resolved in the future.

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